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(71) Applicant: LIPOSOME TECHNOLOGY, INC. [US/US]; 1050 Hamilton Court, Menlo Park, CA 94025 (US).

(72) Inventor: RADHAKRISHNAN, Ramachandran ; 4335 Cambria Street, Fremont, CA 94538 (US).

(74) Agent: DOLEZALOVA, Hana; Phillips, Moore, Lempio & Finley, 177 Post Street, Suite 800, San Francisco, CA 94108 (US).

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(54) Title: A NOVEL NONPHOSPHOLIPID LIPOSOME COMPOSITION FOR SUSTAINED RELEASE OF DRUGS

(57) Abstract

A novel, nonphospholipid liposome formulation for sustained release and delivery of steroids. The formulation provides prolonged release of the drug, improved therapeutic ratio, lower toxicity, reduced systemic side effects and is stable for up to three months. The formulation is suitable for sustained deliverey of steroid via inhalation, parenteral, intrathecal, intraarticular, topical, ophthalmic, and oral administration and is suitable for treatment of inflammatory, arthritic, rheumatoid, topic, pulmonary and interstitial lung diseases.

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A NOVEL NONPHOSPHOLIPID LIPOSOME COMPOSITION FOR SUSTAINED RELEASE OF DRUGS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a novel nonphospholipid liposome composition for efficient loading and sustained release of drugs. The composition is particularly useful in formulating steroids and other drugs for inhalation, and targeted systemic, parenteral, oral, intrathecal, intraarticular, nasal, ophthalmic and topical administrations for human and veterinary therapeutic applications.

Related Disclosures

Sustained release has become a very important feature of modern pharmaceutical sciences. This is particularly true for pharmacologically potent drugs such as steroids. Steroids, in particular corticosteroids, have powerful effects on immunologic and hormonal processes, and are very effective in treating a wide range of inflammatory diseases, such as arthritis, rheumatoid arthritis, allergic reactions, conditions such as asthma, interstitial lung diseases and other lung diseases, and are widely used for topical treatment of ophthalmic and dermatological irritations.

As with many potent drugs, when given systemically the therapeutic benefits of corticosteroids are accompanied by an array of deleterious side effects and complications. Attempts to minimize these complications for example by daily systemic administration of smaller, insufficient and inadequate doses of steroids for desired therapy led to unsuccessful or prolonged treatments, or by administration of higher doses of steroids on alternate days led to uneven levels and peaks of the steroid in the blood level followed

by severe side effects. Both, extended treatment and side effects, are undesirable.

Due to poor solubility of steroids in water, previously used methods of steroids formulation had relied on use of organic solvents or on crystalline suspensions in an aqueous medium. Both crystalline suspensions or solution of steroid in an organic solvents are prone to cause tissue irritation and may be painful when administered by certain routes.

To avoid severe systemic side effects, one of the routes of administration of steroids for treatment of pulmonary conditions is via inhalation. However, the inhalation of steroids formulated in the solvent or as crystalline suspension leads to a rapid absorption with possibility of overdose, irritation and need for frequent dosing when lower doses are used. Notwithstanding, even with these disadvantages, steroidal inhalants are preferable to systemically-administered steroids because they reduce, albeit not eliminate, the side effects when inhaled at recommended doses. The need for repeated dosing and the danger of irritation can only be avoided by providing the formulation allowing for sustained controlled release of the steroid.

The advantage of administration of steroids by inhalation over the systemic administration can best be illustrated by using, for example, a potent antiinflammatory steroid dexamethasone. To achieve desired therapeutic effect, dexamethasone is administered systemically by i.v. injection in doses ranging from 0.5 to 9 mg/day. Where, however, dexamethasone is administered via inhalation, the dose may be decreased to approximately 0.084 mg per one inhalation dose. The total dose of inhaled dexamethasone daily, even when the inhalation is repeated at the maximum dosing frequency, (12 times a day), corresponds to between 0.4 to 1.0 mg of absorbed dexamethasone a day. PDR: 1312 and 1315 (1988). This steroid dose given by inhalation achieves the same therapeutic effect as systemic dose.

Beclomethasone, halogenated synthetic analog of cortisol used in a form of beclomethasone dipropionate (BDP) is often used for inhalation for treatment of bronchial asthma and seasonal and perennial rhinitis. Because beclomethasone dipropionate is poorly soluble in water, it is currently formulated as a microcrystalline suspension in chlorofluorocarbons (Freon) propellants. PDR:1003 (1988).

The advantages connected with using inhalation route rather than systemic administration are lessened by the necessity of multiple dosing. Such dosing is inconvenient, unpleasant, and may lead to nasal or oral mucosal tissue irritation or injury caused by repeated application of a propellant, solvent or other additives necessary for nasal or oral inhalation administration.

One of the life threatening type of pulmonary diseases currently treated with steroids are interstitial lung diseases (ILD). ILD are disorders involving lung parenchyma with different etiologies but similar clinical features and diffuse pathologic changes that affect primarily interalveolar interstitial tissue. They form a heterogeneous group of nearly two hundred diffuse, noninfectious, nonmalignant, inflammatory, and often fatal disorders of the lower respiratory tract, resulting in pathological changes of alveolar tissue, in particular alveolar septum, epithelial and endothelial cells. These diseases progress from the initial acute stage through semichronic to chronic stage and are characterized by progressive development of extensive lung fibrosis or granulomatosis at a later stage of interstitial fibrosis, to a progressive destruction of the lung and formation of cysts interspersed with thick bands of fibrotic tissue, so called honeycomb lung. At this stage, the lung tissue is remodeled and reorganized to such an extent that the airway alveolar structure is lost and replaced with irregular air spaces with fibrotic walls. Pathol. Annals, 21:27 (1986).

Current therapy of ILD includes systemic administration of

multidoses of steroids, in particular corticosteroids or glucocorticoids alone or in combination with other drugs. Most often used therapy for ILD is 40-80 mg/day of prednisone orally for one to two months. To control symptoms in many ILD chronic cases, a follow-up treatment with lower doses (5-15 mg/day) is needed for weeks, years, or indefinitely. Still, favorable responses to such massive doses of steroids are achieved in only 20-60% of patients. (The Merck Manual, 14th Ed., p. 260 and 685 (1982); Clin. Geriatr. Med., 2:385 (1986); J. Resp. Dis., 10:93 (1989).

Massive doses of steroids, while beneficial and tolerable for a short period of time, are accompanied by severe side effects and the benefit of long-term treatment with steroids may be thus lessened. Moreover, steroids formulated for inhalation seem to be rapidly absorbed in upper respiratory regions with very little, if any, of the steroid ending up in alveoli, a primary area affected by the inflammation leading to ILD.

Thus it would be desirable to provide an inhalation formulation which would deliver steroid and or other drug in sustained, time released fashion into the lower lung region.

For successful delivery of drugs into alveoli of the lower pulmonary region, it is important to eliminate from the formulation irritants such as chloroflurocarbons, to decrease the number of required doses, and to provide vehicles that allow deposition of steroid in the alveolar region. Such need can only be met by providing aerosol droplet particles with a mass median aerodynamic diameter of approximately 1-2.1 μ size with a geometric standard deviation (GSD) of 1μ. Providing sustained controlled release of the drug from such aerosol would be an added benefit. With the size requirement as outlined above for particle aerosol droplets, presized liposomes of approximately 0.2μ or micelles of particle size of approximately 0.02μ, can be used for the generati n of aerosol particles that can be deposited in the alveoli in

significant amount.

Because of the specific requirements of aerosol droplets of micron or submicron sizes needed for inhalation therapy of the ILD, such therapy has not been until now available. Thus it would be highly desirable to have available a steroidal formulation suitable for inhalation which would provide small, substantially homogeneous size aerosolized particles allowing the steroid or other drug to be deposited in the alveoli.

Previously disclosed (EP 87309854.5) small particle aerosol liposomes and liposome-drug combinations for medical use tried to circumvent, but fell short of, the strict size requirement for delivery of steroid into alveoli. With aerosol particle size requirement for deposition in alveoli around 1-2.1 μ mass median aerodynamic diameter (MMAD), the size of aerosol droplet delivering drug into alveoli must be substantially within that size limit, preferably with the majority of single aerosol droplet about or smaller than 2 μ for optimal alveolar deposition. The above cited reference attempted processing a heterogeneous size (1-10 μ) population of liposomes into a more homogenous size of smaller liposomes using an aerosol nebulizer equipped to reduce the size of liposomes. In this manner, the majority of resulting aerosol particles were less than 5 μ in diameter with an aerodynamic mass median diameter ranging from about 1-3 microns. Although some of these particles may reach alveoli, a sizable fraction is far too large to be able to enter the small alveoli and consequently, the drug payload in deep lung is therapeutically insignificant. Also, because of the sizing by aerosolization, the size distribution of these liposomes is unpredictable and the amount of drug deposited in the deep lung cannot be estimated or predicted with any degree of certainty.

Other important routes of steroids' administration are intraarticular injection of steroid into inflamed joints and intrathecal injection of steroids into the brain and spinal

cord during bacterial, inflammatory and viral diseases of the central nervous systems, nasal or oral administration during bacterial, viral or allergic reactions or cold symptoms, topical administration during dermatitis or bacterial infections, various parenteral administrations such as intravenous, intramuscular, intraperitoneal, subcutaneous or percutaneous for treatment of all kinds of infections, inflammations and allergic conditions.

All these routes of administration encounter the same problems as outlined above. Either the doses of administered steroid are too large causing unwanted side effects or too low being insufficient for effective treatment of conditions needing treatment. Moreover, since some of these routes of administration are extremely painful and unpleasant, for example intrathecal or intraarticular injections, it would be of great advantage to have a steroidal formulation allowing sustained release of the drug which would eliminate a need for frequent and repeated injection or other dosing.

Moreover, due to steroids poor solubility in aqueous systems it is necessary to add to steroid formulations solubilizing agents such as ionic surfactants, cholates, polyethylene glycol (PEG), ethanol, and other solubilizers or use micronized suspension of crystalline drug. While in general these agents are considered pharmaceutically acceptable excipients, many of them have undesirable side effects particularly when used in inhalation, parenteral, intraarticular, intrathecal, nasal or topical formulations. The deleterious effect of agents such as PEG in membrane permeabilization and local irritation is well documented.

Therefore, it would be advantageous to provide steroid formulations without the necessity of adding such solubilizing agents and be able to provide for a sustained timely released supply of the drug to the diseased organ or site of infection including deep lung.

Certain improvements have previously been achieved by encapsulating steroids in conventional phospholipid liposomes. For example, smaller doses of steroids were found to be effective when administered in liposome encapsulated form and modest prolongation of effect and restriction of the drug to the site of administration was achieved with marginal degree of decreased systemic uptake.

Liposome drug delivery systems are reviewed in detail in Cancer Res., 43:4730 (1983). In general, liposomes are advantageous in that they can provide and improve controlled release of an entrapped drug, reduce side effects by limiting the concentration of free drug in the bloodstream, alter the tissue distribution and uptake of drugs in a therapeutically favorable way, and make therapy safer and more convenient by reducing the dose or frequency of drug administration. Decreased toxicity and degradation, use of smaller doses, a targeting the liposomes toward a specific site, and reducing side effects of a liposome-bound steroid over the use of a free or polymer-bound steroid have been described in Nature, 271: 372-373 (1978).

By providing a stable drug suspension, the use of liposomes as a solubilizing agent for steroids or other drugs in aqueous, nebulized inhalation suspensions essentially eliminates the use of potentially toxic halogenated hydrocarbon propellants and co-solvents, and prevents irritation caused by drug sedimentation and crystallization often encountered with conventional steroidal suspension.

Notwithstanding the above, utilizing liposomes for inhalation still faces numerous problems. For example there is a little or no effect of liposomal entrapment on rapid systemic uptake, which remains unchanged indicating that even from the liposomes, the drug is still rapidly released.

Because of their poor formulating properties, many useful steroids had to be derivatized or modified in order t be accommodated within the chemical structure of the

liposomes for enhanced retention. For example, 6-18 carbon-chain ester needs to be present in the steroid molecule for optimal lipophilic interaction between a water-insoluble corticosteroid a the lipid membrane. Due to phospholipid liposomes inability to provide a strong enough barrier to slow down the release of the derivatized steroid and to achieve prolonged release steroid derivative still did not slow the release rates of steroid from liposomes.

U.S. Patent 4,693,999 discloses new steroid derivatives obtained by modification of corticosteroids with fatty acid esters. These modified steroids incorporated in the lipid portion of liposomes for delivery via inhalation provide prolonged steroid retention in the respiratory tract, however, designing and synthesizing new steroid derivatives is inconvenient, costly, slow, laborious and often changes the drug efficacy.

Water-insoluble steroids are generally difficult to load into conventional phospholipid liposomes because these molecules tend to crystallize rather than incorporate into the liposomal membrane. Such drug crystallization causes the same sedimentation problems and free drug toxicity upon administration as do nonliposomal steroidal suspensions. Modified steroids, unlike cholesterol which is ubiquitously distributed in biological membranes, in particular seem to be structurally or sterically incompatible with phospholipids in terms of hydrophobic or Van Der Waals interactions and thus crystallize out readily.

Previously available conventional liposomal drug formulations have shown an uncontrollable and impractically fast release rate. Measurements of systemic uptake from the respiratory tract after inhalation of underivatized steroids or other drugs formulated in conventional liposomes indicated little or no effect of liposomal entrapment on the release rate. This means that despite the liposome-binding, the drug was still released relatively quickly from the c nventional ph spholipid liposomes. In case of steroids,

this was probably due to their lipophilic nature which causes the steroid to be release from the lipid membrane faster than water-soluble drugs encapsulated in the liposomes. Biochem J., 158:473-6 (1976).

As may be seen from above, many problems remain unresolved with steroid or other drugs formulations using conventional phospholipid liposomes. Some of these problems relate to the requirement for drug modification, poor drug loading into liposomes and poorly controlled release rate.

It is the primary object of this invention to provide a nonphospholipid liposome composition wherein the poorly water soluble, sedimentation-prone, underivatized or unmodified steroids or other drugs are successfully sequestered within the liposomal lipid vesicles of uniform and controllable particle size, having high encapsulation values, long-term stability, and effective sustained release with a controllable potency of the drug. The resulting composition will allow an administration of low doses of steroid or other drugs thus reducing toxicity and systemic side effects and provide pharmacologically bioavailable doses of the drug in situ.

SUMMARY

One aspect of this invention is to provide nonphospholipid liposome based formulation comprising cholesterol, cholesterol ester salt and underivatized and unmodified steroidal or other drug for therapeutic delivery.

Other aspect of this invention is to provide formulation enabling high efficiency liposome entrapment of underivatized steroids and other drugs in the liposome vesicles of uniform and controllable particle size.

Yet another aspect of this invention is to provide liposome composition which has lower toxicity, lower side effects, allows the targeting and release of the drug at a site of specific organ, removes need for multiple dosing, can be sterilized, and is sufficiently stable in dried form for long-term storage.

Another aspect of this invention is to provide controlled, sustained release of the steroidal and other drugs from the nonconventional liposome/drug composition.

Yet another aspect of this invention is to provide liposome/drug compositions which has lower toxicity, lower side effects, allows the targeting to and release of the drug in a deep lung tissue and removes need for multiple dosing.

Yet another aspect of this invention is to provide the method of treatment of interstitial lung diseases by administering the nebulized liposomal drug composition by oral inhalation.

Still another aspect is to provide a process for making novel nonconventional liposome composition for controlled sustained release of steroidal or other drugs.

Still yet another aspect of this invention is to provide the method of use of the nonphospholipid liposomal drug compositions for delivery by inhalation, intratracheal, peroral, parenteral, such as intravenous, intraperitoneal, intramuscular, or subcutaneous, percutaneous, topical, intraarticular, intraventricular and ocular routes of administration.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the rat plasma concentration of BDP after intravenous injection of radiolabeled BDP.

Figure 2 shows the rat plasma concentration of BDP after intratracheal instillation of radiolabeled BDP.

Figure 3 shows the rat plasma concentration of BDP after intratracheal instillation of radiolabeled BDP encapsulated in two types of conventional liposomes.

Figure 4 shows the plasma kinetics of radiolabeled BDP after intravenous administration of free BDP and intratracheal instillation of radiolabeled BDP encapsulated in conventional cholesterol containing liposomes.

Figure 5 shows the amount of plasma BDP radioactivity for two hours following the intratracheal instillation of

nonconventional liposomal BDP illustrating sustained release and for three hours following the administration of free drug.

Figure 6 shows the amounts of radiolabeled BDP remaining in the rat lungs following intratracheal instillation of five different liposome-encapsulated BDP formulations and the amount of the radiolabeled BDP in the lungs found after the intravenous administration of the free BDP.

Figure 7 shows the plasma concentration of free BDP and BDP encapsulated in nonconventional liposomes and the sustained release of liposome encapsulated BDP versus a total BDP.

Figure 8 depicts pulmonary anatomy showing the division of one larger bronchus into smaller bronchi, bronchioli, terminal bronchioles, respiratory bronchioles, alveolar ducts, sacks, and ultimately into individual alveoli.

Figure 9 depicts the current concept of pathogenesis, clinical symptoms and pathological changes connected with interstitial lung diseases.

Figure 10 is a diagram for nebulization of a steroid liposome suspension and collection of aerosol output on Anderson cascade impactor stages corresponding to the human respiratory system.

Figure 11 depicts Andersen's Sampler as a simulator of a human respiratory system.

Figure 12 shows the mass median aerodynamic diameter and aerosol particle size distribution of BECOTIDE®.

Figure 13 shows the mass median aerodynamic diameter and aerosol particle size distribution of liposomal beclomethasone dipropionate.

DETAILED DISCLOSURE OF THE INVENTION

Preparation Procedures

According to the present invention, it has been discovered that beclomethasone dipropionate, other steroids in underivatized form and other drugs may be successfully retained in nonconventional liposomes for sustained release

when the liposomes are formulated to contain a mixture of cholesterol and cholesterol ester salt such as for example sodium cholesterol sulfate, also known as cholesteryl sodium sulfate (cholesterol sulfate). Sodium cholesterol sulfate or other cholesterol ester salts act as a temporary barrier against drug efflux from the liposomes. To design the optimal formulation for high drug loading and sustained release of underivatized steroid, a number of different formulations were developed.

Methods of Liposome Formation

The liposome suspension of the invention can be prepared by any of the standard methods for preparing and sizing liposomes. These include hydration of lipid films, solvent injection, reverse-phase evaporation, dehydration rehydration, freeze thaw and other methods, such as those detailed in Am. Rev. Biophys. Bioeng., 9:467 (1980). Reverse-phase evaporation vesicles (REV) prepared by the reverse-evaporation phase method is described in U.S. Patent No. 4,235,871, incorporated hereby by reference. The preparation of multi-lamellar vesicles (MLV) by thin-film of a lipid film or by injection technique is described in U.S. Patent 4,737,923, incorporated by reference. In two later procedures, which are generally preferred, a mixture of liposome-forming lipids dissolved in a suitable solvent is evaporated in a vessel to form a thin film, which is covered by an aqueous buffer solution. The lipid film hydrates to form MLVs, typically with sizes between about 0.1 to 10 microns.

Either the REV or MLV preparations can be further treated to produce a suspension of smaller, relatively homogeneous-size liposomes, in the 0.1-1.0 micron size range. Advantages of smaller, more homogeneous-size liposomes are, for example the higher density of liposome packing at a mucosal tissue surface, the higher concentration of liposome encapsulated drug transported to the target organ or tissue, or the greater optical clarity

The use of all SUV, LUV, MLV, OLV or mixture thereof is contemplated depending on intended therapeutic application and route of administration.

One preferred method for producing SUV is by homogenizing an MLV preparation, using a conventional high pressure homogenizer of the type used commercially for milk homogenization where the MLVs are cycled through the homogenizer, and periodically sampled for particle sizes to determine when the MLV have been substantially converted to SUVs.

The drug is encapsulated in the liposomes by using for example the procedure described in U.S. patent 4,752,425, incorporated by reference.

Conventional and Nonconventional Liposomes

As defined herein "the conventional liposomes" mean liposomes which contain phospholipids, and the "nonconventional liposomes" mean liposomes which do not contain phospholipids but are formed solely by cholesterol and cholesterol derivatives or, in alternative by amphipathic lipid components.

Both conventional and nonconventional liposomes can be formed by a variety of standard methods from a variety of vesicle-forming lipids. For the conventional liposomes these lipids include dialiphatic chain lipids, such as phospholipids, diglycerides, dialiphatic glycolipids, and cholesterol and derivatives thereof. The various lipid components are present in an amount between about 40-99 mole % preferably 60-90 mole % of the total lipid components in the liposomes, cholesterol or cholesterol derivatives are present in amounts between 0-40 mole %. In the nonconventional liposomes the cholesterol derivatives are present in amounts between 30-70:20-50:0.01-20 mole % of cholesterol derivative to cholesterol to drug, respectively. The drug encapsulated in both kinds of liposomes is in amounts of 0.01-20 mole %.

liposomes demonstrate higher drug loading with encapsulation values of 100%, when 10 mole % dose is used (total lipid concentration of 40 umol/ml) compared with conventional phospholipid liposomes. These liposomes generally allow only about 1 mole percent drug encapsulation at a total lipid concentration of 40 umol/ml. For example, unsaturated conventional liposomes without cholesterol have the flexibility of accommodating only 1 mole percent of steroidal drug and their encapsulation value is therefore very small. Saturated conventional liposomes composed of lipid such as fully hydrogenerated soy PC do not accommodate even small amounts of the steroidal drug. Moreover, large amount of crystalline steroid could be detected to be present in these conventional liposomes after extrusion and on storage. Even though lyso PC containing liposomes can accommodate a steroid to fill in the acyl chain vacancy, such liposomes exchange and release their drug readily, and rapidly defeating thus the whole purpose of drug encapsulation in liposomes. (Table I).

Example 1, Table 1, illustrates the % encapsulation of steroid in the various conventional and nonconventional liposomes. While the % encapsulation for some conventional liposomes may seem high, the actual amount of drug which can be encapsulated in conventional liposomes (A-H) is about 10% of the amount of steroid which can be encapsulated in nonconventional liposomes (I-L).

Stability

Stability problems are also overcome in a current nonconventional liposome formulation, in terms of the sedimentation and crystallization problems encountered with nonliposomal or conventional liposome suspensions. Because of the unique cholesterol sulfate formulations which accommodate the drug by steric fit, and because of their high encapsulation and high retention values, drug crystallization does not occur outside or inside the liposomes, nor do s sedimentation occur from the suspension.

heated air, and typically about 30°C. The dried particles are collected and stored as a powder in dehydrated form, under an inert atmosphere in the presence of a desiccant. Such powders are storable under these indications for at least a year at ambient temperature. Dry powder liposomes can be used as injectable materials after reconstitution or suspended in appropriate dilutants or freon propellants for aerosol administration or formulated to topical, nasal or oral dosage forms.

Method of Preparation of Surfactant Micelles

Alternatively, steroids may be solubilized in surfactant micelles and nebulized into small aerosol particles by using appropriate nebulizers. Typical mixed micellar formulations of steroid contain an appropriate surfactant detergent such as sodium methyl cocoyl taurate (Tauranol® WS) obtained from Finetex, N.J., cholate or deoxycholate, polysorbate 20, or polyoxyethylene sorbitan monolaurate (Tween® 20) obtained from Sigma or poloxamer (Pluronic® F68 Prill) obtained from BASF Wyandotte Corp. N.J., in amount from 1-100 mg per ml, preferably between 40-60 mg/ml, mixed with steroid drug in amounts from 0.1-20 mg/ml, preferably in amount 0.2-1 mg/ml. The weight ratio of surfactant to drug is from 100-200:0.2-10, preferably around 155:1. The mixture is let stand under stirring for 2-48 hours, preferably overnight at temperature between 16-40°C, preferably at ambient temperature. Then the mixture is filtered over filter with pore sizes smaller than steroid crystals, usually using 0.1-1μ filter. Filter, on which the undissolved drug is deposited, is discarded and the micelle filtrate is used for nebulization as described below.

Micelle is the term used to describe the suspension of surfactant in water. In a micelle-steroid drug suspension, drug is intercalated between two layers of surfactant with polar group being situated on outside. pH of micelles varies and maybe from around 4.25 to preferably around 7.4-7.8. Additionally, other additives, such as saline, mono or

dibasic sodium phosphate may be added in amount to reach and/or maintain osmolality of the mixed micelles between 200-500, preferably around 300 mOsm/kg. The micelles are prepared in deionized distilled water to make up volume wherein per each ml there is present surfactant, steroidal drug, saline or other salt in amount to fall within ratios given above, preferably about 60 mg/ml surfactant; 0.4 mg/ml of drug and 9 mg/ml of saline.

While the use of micelles as particle aerosol useful for treatment of interstitial lung diseases is contemplated to be within the scope of this invention, the loading of drug into micelles and the sustained release of drug are limited.

Aerosolization or Nebulization of Liposome Formulation

Since interstitial lung diseases are primarily diseases of the deep lung, the delivery of corticosteroids and other drugs used for treatment of alveolar inflammation to the site of the inflammation is of primary interest. Focused administration of steroids or other drugs to the lung parenchyma via oral inhalation represents an attractive alternative to the oral route for the treatment of ILD and offers the potential to concentrate the drug at a site where it is needed while minimizing systemic absorption and accompanying side effects. Solubilization of steroids in an aqueous formulation and subsequent generation of small aerosol droplets by nebulization are important prerequisites toward achieving this goal. Several inhalation dosage forms of steroid drugs have been previously developed for the treatment of bronchial asthma. However, due to their inherent insolubility, steroid preparations could only be formulated as propellant suspensions, such as for example Freon 11-clathrate suspended in Freon 12/114 mixture or as aqueous suspensions with surfactants. These suspensions, which are administered by nebulization or by using propellant-based meter dose inhalation systems, are not amenable to the generation of small particle aerosols of the type required for deep lung penetration. As has been shown

in the parent application, U.S. Serial No. 284,158, filed on December 14, 1988, steroids may be advantageously formulated in nonconventional i.e., nonphospholipid liposomes. Similarly, steroids may be formulated in surfactant micellar solutions. Steroids solubilized in either of these entities are able to be nebulized using appropriate nebulizers to form small particles with good drug output as described above. Nonconventional liposomes offer several advantages including greater loading efficiencies and safety. For example, nonconventional cholesterol sulfate liposome are able to incorporate around 2 mg or more of drug per ml of solution used for nebulization, generating aerosol droplets with a mass median diameter between 0.4-0.9 μ. Since the size of the aerosol droplets reaching alveoli is assumed to have MMAD 0.02-2.1 μ, the aerosol droplets generated by the method described below, are able to be deposited, upon inhalation, in the deep lung of alveolar tissue.

Pharmaceutical aerosols of this invention are suspensions of nonconventional liposomes or micelles containing steroid, preferably beclomethasone dipropionate in as large amounts as can be possibly formulated. For nonphospholipid liposomes, these amounts are from 0.1 mg/ml to about 2 mg/ml of suspension. For micelles, the suspended amount of steroid in surfactant, preferably Tauranol WS, is about 0.4 mg/ml.

Liposomes or micelles are prepared as describe above. Liposomes are presized to contain substantially homogeneous liposome population with a mean particle size of 0.2 μ. The liposomal or micellar suspension is placed in the nebulizer and, as illustrated in Figure 10, the air compressor is attached to the lower part of the nebulizer at point B. By the pressured air generated from the compressor, the solution in the nebulizer is agitated into a mist of aerosolized particles droplets of sizes predominantly between 0.02-3 μm with an MMAD not exceeding 2.1 μm. These particles are then moved to the connecting tubing having

inserted one-way valve with filter. The aerosol particles move toward the mouthpiece to be used for a patients' inhalation. Larger particles fall back to nebulizer and again undergo aerolization. In the real life situation, expired air carrying very small particles may be trapped in the air filter provided.

In practice, the nonconventional liposome steroidal suspension or micellar solution preformulated in the concentration and amount as described above (or the formulation may be sufficiently diluted with sterile saline or a suitable diluent to known concentration of active ingredient) is poured into the nebulizer, the nebulizer is connected to the air compressor, and the patient inhales via a mouth piece the aerosolized suspension.

Figure 10 represents a model for studying a nebulization of steroid suspension on the Anderson cascade impactor stages. The principle of the model is that the impactor is divided into Stages 0-7, having segments separated from each other by the stages with pores 10 μ and above-preseparator stage, 9-10 μ - Stage 0; 5.8-9 μ - Stage 1; 4.7 - 5.8 μ - Stage 2; 3.3 - 4.7 μ - Stage 3; 2.1 - 3.3 μ - Stage 4; 1.1 - 2.1 μ - Stage 5; 0.65 - 1.1 - Stage 6; and 0.43 - 0.65 - Stage 7. A suitable filter is placed at the end to collect any submicronic droplets. As can be seen from Figure 11, only Stages 5, 6, 7 and filter correspond to droplets of 0.4 to about 2.1 μ (MMAD) reaching alveoli. Consequently, only aerosol particles which pass Stage 4 into Stages 5, 6, 7 and submicronic filter are useful for delivering drugs into alveoli.

Aerosolization of nonconventional liposomal suspension or micelles produces droplets containing the expected amount of steroid, i.e., around 1.7-2 mg/ml of aerosolized solution for liposomes and 0.4-0.5 mg/ml of aerosolized micellar solution with a mass median aerosol diameter of 0.4 - 0.9 μ. A majority of the aerosol particles were found in stages 5, 6 and 7 of the impactor and may be delivered into alveoli.

Figures 12 and 13, compare the alternative aqueous steroidal suspension of BECOTIDE® (Figure 12) to a liposomal beclomethasone dipropionate Formulation (Figure 13).

Figure 12 shows the liquid aerosol particle size distribution of BECOTIDE® generated using an ultravent nebulizer with pulmoaide compressor pump mass distribution being done by QCM impactor with an isokinetic flow divider. As can be seen, 50% of all particles generated from liquid BECOTIDE® suspension have an effective mass median aerodynamic diameter (MMAD) of 2 μ. MMAD is Stokes Diameter described in An Introduction to Experimental Aerobiology, p. 447, Wiley (1966) and is an equivalent mean diameter. When in the same experimental set-up, the liposomes containing 2 mg/ml of beclomethasone are aerosolized, 50% of all particles have MMAD around 0.4 μ. Only 15% are larger than 2 μ, with 50% equal or smaller than 0.4 μ.

Andersen cascade impactor is obtained from Andersen Air Sampler Inc., Atlanta, GA; QCM Cascade impactor is obtained from California Measurements, Sierra Madre, CA. Single-use ultravent nebulizer is obtained from Mallinckrot, St. Louis, MO, and Respigard II nebulizer is obtained from Marquest, Englewood, CO.

Parameters followed for aerosolization were percent of drug recovery, nebulization or aerolization rate, MMAD, percent alveolar deposition relative to total nebulizer volume and analyses of fractions in nebulizer, throat, Y-joint, stages and down stream submicronic filter.

In order to determine the rate of absorption of the steroidal drug into the plasma after intratracheal administration, various formulations containing either the free steroid or steroid encapsulated in liposomes were prepared and tested. Free steroid drug, in this case ^{14}C labeled BDP, dissolved in ethanol/water (1:1) was administered to rats either intravenously (Figure 1) or intratracheally (Figure 2).

The free drug control was administered intravenously to provide relative basis for measurement of bioavailability. Blood samples were taken at 5, 10, 20, 40 60, 90 and 120 minutes and the radioactivity of radiolabeled BDP was determined using standard scintillation counting technique. The resulting plasma profiles, given in Figure 1, illustrate the physiological removal of steroid from the blood circulation. In order to determine the plasma uptake of free steroid from lungs following the intratracheal instillation of radiolabeled ^{14}-C BDP, the same free drug formulation was instilled into rat lungs and the blood samples collected at intervals of 5, 10, 60, 90, 120, 150, 180 minutes. As will be seen from Figure 2, the rate of absorption of free steroid from lungs to the plasma is rapid and the physiological removal from the plasma follows the same course as that of the free drug. When a similar experiment was performed with radiolabeled ^{14}C-BDP encapsulated in conventional anionic liposomes (EPC/EPG/BDP; 96:3:1) or in conventional neutral liposomes (EPC/BDP; 99:1), the rate of absorption was also rapid for both formulations (Figure 3). Thus, the rate of absorption from lungs to plasma of free steroid and steroid encapsulated in conventional liposomes is not much different and follows similar curve.

The pharmacokinetic parameters of free radiolabeled ^{14}C-BDP (0.008 mg/kg in 50% ethanol) administered intravenously to a group of 12 rats, and intratracheally instilled radiolabeled BDP (0,007 mg/kg) encapsulated in conventional liposomes (EPC:cholesterol sulfate: BDP;32:9:65.8:1.3) is illustrated in Figure 4. The plasma kinetics of both free and encapsulated BDP in conventional liposome containing cholesterol sulphate and phospholipid is virtually identical, indicating that BDP is rapidly and completely absorbed from the lungs after intratracheal instillation of drug containing conventional liposomes.

The delayed and/or sustained release of the steroid from the nonconventional liposome formulation containing combination of cholesterol/cholesterol sulphate and the steroid is shown in Figure 5. Figure 5 shows the plasma radioactivity of ^{14}C-BDP following intratracheal instillation of free ^{14}C-BDP and intratracheal instillation of ^{14}C BDP encapsulated in nonconventional liposomes. While the free BDP is quickly removed from the lungs into plasma and metabolically eliminated, the rate of release of the liposomal BDP into the plasma is much slower. The concentration of ^{14}C-BDP in plasma initially increases, probably due to presence of some percentage of free BDP. Subsequently, it reaches and maintains certain plasma level equal to the rate of metabolic removal. In other words, after the first thirty minutes, the near equilibrium is reached in that the liposomal formulation releases only that much of the BDP into the plasma as is eliminated. Moreover, the nonconventional liposomes are able to sustain that level for measurable time. Pharmacokinetic properties of the steroidal drug are thus altered by drug incorporation into these liposomes.

Sustained release of four nonconventional liposome formulations, containing sodium cholesterol sulfate/cholesterol/^{14}C-BDP in various ratio, namely 50:40:10 mole % with 0.260 mg/kg of BDP; 55:40:5 mole% with 0.260 mg/kg of BDP; 53:37:9 mole % with 0.187 mg/kg of BDP; and 50:40:10 mole % with 0.035 mg/kg of BDP was compared with the free BDP administered intravenously and with one formulation of conventional liposomes containing sodium cholesterol sulphate/egg phosphatidylcholine/^{14}C-BDP in ratio of 30:60:1.2 mole % with 0.007 mg/kg of BDP (Figure 6).

Linear plots were obtained when the amount of radiolabel remaining in the lungs was plotted against time on semi-log paper, indicating that all four formulations were absorbed from the lungs by a first order pr cess. These data were

fit by single exponential functions using a non-linear least squares curve fitting program (RSTRIP). The resulting slopes and intercepts were used as estimates of the absorption rate constant (K_a) and the amount of drug in the lungs at zero time, respectively. The absorption rate constants for the four cholesterol/cholesterol sulfate formulations ranged from 0.64 hr^{-1} for , 0.74 hr^{-1} for 0.84 hr^{-1} for to 1.03 hr^{-1} for corresponding to an absorption half-life of 0.68 hr, 0.78 hr, 0.89 hr, to 1.09 hr, demonstrating that sustained in vivo release of liposome-incorporated BDP had been achieved. The apparently longer half-lives for free ^{14}C-BDP (3.0 hr) and EPC/CH (2.4 hr) formulations shown in Figure 6 are clearly not absorption half-lives since over 98% of the drug was absorbed before the first time point. These later values relate to the elimination of radiolabel already released from the liposomes and distributed to the lungs. The amount of drug in the lungs at time zero can be used to determine the amount of free drug in the formulation, since free drug is very rapidly absorbed from the lungs (Dose = free drug + amount in lungs at t=0). This amount also includes any liposome associated drug that was rapidly released ("burst" effect). The amount of drug present in the lungs at time zero (T_0) varied among formulations and was 90 - 48% for these nonconventional liposomes, although in vitro measurements by membrane exchange assay did not detect any free drug in the formulations. This would indicate that there are rapidly and slowly released pools of drug within each liposomal formulation.

The absorption kinetics (sustained release) was determined by measuring of percentage of ^{14}C BDP remaining in the lungs following the intratracheal instillation of the above described five liposome formulations and one intravenous administration of free drug. In less than thirty minutes, 99.7% of free ^{14}C-BDP was removed from the lungs and 98.8% of th BDP encapsulated in conventional

liposomes. In contrast, only 20% of radioactivity of ^{14}C BDP encapsulated in the best nonconventional liposomes was removed from the lungs with 23% of radioactivity still being present at 180 minutes. The other three nonconventional liposome formulations also should sustain release of the steroid for the same time. Thus, the presence of cholesterol in combination with cholesterol salt and the absence of phospholipids is essential for sustained release of the steroid from the nonconventional liposomes.

Corresponding plasma concentration versus time data (set up in Figure 5) were obtained for one of the nonconventional sustained release formulations listed in Figure 6. The plasma concentration versus time curve observed after administration of ^{14}C-BDP (0.187 mg/kg) in a cholesterol/cholesterol sulfate liposome formulation was strikingly different from that of free drug, remaining nearly flat over the two hour duration of the study (Figure 5). Since lungs data indicated that 27% of the administered dose was free or rapidly released drug, the plasma concentration curve of Figure 7 reflects the sum of concentrations due to "free" and "encapsulated" drug. The concentration time curve for "free" drug was estimated by assuming 27% of the dose was immediately absorbed and followed the kinetics observed for i.v. administration of free BDP. This curve was subtracted from the experimentally observed data to give an estimate of the plasma concentration due to liposomal sustained-release BDP (Figure 7). It is clear that the plasma concentration versus time curve for the cholesterol/cholesterol sulfate formulations differ substantially from those observed following i.v. and conventional EPC liposome administration of BDP (Figure 4).

In order to determine whether BDP was absorbed as unchanged drug or metabolized prior to release and absorption, lung samples from one study were analyzed by a thin layer chromatographic assay capable of separating BDP from its monopropionate hydrolysis products. The result

showed no detectable metabolism of BDP prior to leaving the lungs.

The cholesterol ester salt and cholesterol are mandatory components of the nonconventional liposome formulation and are not interchangeable with a phospholipids, normally used in conventional liposome compositions. The cholesterol is primarily responsible for, and greatly affects the sustained release, but the in vivo drug-release half life depends on the relative amount of cholesterol sulfate and on the absolute presence of cholesterol in the composition. Drug release half life can be varied accordingly. For example, liposome composition containing egg phosphatidyl choline: cholesterol sulfate:BDP (60:30:10) has a drug-release half life in vivo only slightly lower than the conventional liposomes without cholesterol sulfate salt or the free drug, but it has pronounced drug retention in vitro compared to compositions without cholesterol sulfate (Example V and Table II). However, nonconventional liposome compositions containing sodium cholesterol sulfate:cholesterol: BDP, (50:40:10; 55:40:5; 53:37:9 mole %) gave markedly delayed release in vivo of the drug when instilled in the respiratory tract of an experimental animal together with having much prolonged drug retention as compared to the retention of the free drug and conventional liposomes (Figure 6).

The plasma kinetics observed following the i.t. instillation of ^{14}C-BDP (0.007 mg/kg) incorporated into conventional EPC:$CHSO_4$ liposomes (Figure 6) were virtually identical to those observed following the i.v. administration of a similar dose of free drug (Figure 4). These data indicate that BDP formulated in conventional liposomes is rapidly and completely absorbed from the lungs after intratracheal instillation of this formulation. This is due to the incompatibility of the physical and molecular nature of the drug and bilayer architecture formed with phospholipids.

Therapeutic Applications

Therapeutic applications and advantages of the nonphospholipid liposomes are numerous. Sustained release of the steroid from the nonconventional liposomes prolongs a therapeutic activity after each administration, reduces the frequency of administration, further improves the ratio of localized-to-systemic effects, and provides increased and extended local therapeutic effect in the lungs, deep lung, joints, brain, spinal cord, blood, muscles, skin, mucosal tissue, eye, and other organs or antineoplastic effect on tumors.

In addition, a sustained release formulation will reduce the amount of drug absorbed by the oral mucosa (due to the salivary action which would more likely clear aqueous liposome suspension into G.I.) thus reducing the incidence of oral or mucosal tissue infection following inhalation, nasal or peroral therapy. Topically administered to the skin of, for example lupus erythematosus patients, or patients with dermatitis or skin allergy, or topically administered to the eye for treatment of allergic and inflammatory conditions the formulation of current invention will provide a long term slow release of the steroid at a place where it is immediately active without need of having the whole body affected by the excess of steroid given systemically or without the need of readministration. Intravenously, intramuscularly or intraperitoneally it will provide a circulating reservoir of the drug to be released slowly to the blood stream and/or in the vicinity of the diseased, inflamed or infected organ or tumorous growth. Intratracheally, the formulation will be delivered into the airways from which it can be slowly and continuously released to the bloodstream and can treat the inflammation of the airways, bronchitis, pneumonia, or tracheal allergic or infectious or inflammatory infections and conditions. Administered in a special formulation suitable for delivery of steroids into alveoli located in deep lung, it will treat

idiopathic infiltrative diseases of the lungs by delivering minuscule liposome droplets into these diseases alveoli. Intrathecally, the formulation will deliver the steroidal drug into the brain, to the central nervous system and spinal cord. Such delivery is important for treatment of acute, subacute, or recurrent meningitis, encephalitis, aseptic meningitis, brain abscesses or spinal cord neoplasms. Intraarticularly, the formulation will be injected into joints for treatment of arthritis, gout, Lyme arthritis, osteoarthritis where the systemic cortiscosteroidal treatment is specifically contraindicated but intraarticular injections are beneficial albeit unpleasant and uncomfortable. The antiarticular injection of the nonconventional liposome steroidal formulation will avoid repeated injections because the formulation will be able to release the needed amount of steroid from the liposome for prolonged period of time.

In one aspect of this invention, spray dried or lyophilized liposomes containing steroid are diluted with 0.9% sterile saline and the suspension placed, after mixing, in a Mallinckrot Ultravent nebulizer and the aerosol is breathed until there is no more liquid in the nebulizer. A typical volume of nebulized solution, deliverable over 10-30 minutes time period is 1-2 ml. Consequently, the ideal aerosolized liposome-steroid suspension contains from 0.2-2 mg of steroid per ml of the nebulized solution. With the loading capacity of nonconventional liposomes being around 2 mg/ml, one inhalation dosage daily is sufficient to provide a daily needed dosage of steroid for treatment of interstitial diseases of lung. However, the dosage with the same, larger or smaller amounts of the drug may be administered to a patient according to a treatment regimen prescribed by a physician.

The examples for providing the data and evaluating the novel composition in this application use primarily the antiinflammatory steroid beclomethasone dipropionate,

cortisone or hydrocortisone with inhalation, intravenous, intrathecal, intraarticular or topical routes of administration. The scope of the invention is not limited to BDP, cortisone or hydrocortisone as a steroid, nor the described routes of administration, but are intended to include all other steroids named below and all other routes of administration.

The invention is applicable, more broadly, to all steroids such as dexamethasone, aldosterone, betamethasone, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, estrogenes, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamicinolone, testosterone or their respective pharmaceutically acceptable salts or esters.

Pharmaceutically acceptable salts refer to salts such as chloride, bromide, iodide, sulfate, phosphate, nitrate, acetate, propianate, glycolate, pyruvate, oxalate, malate, maleate, malonate, succinate, cimamate, mendelate, salicilate, sulfonate, and the like.

Pharmaceutically acceptable esters are esters such as for example metyl ester, etyl ester, butyl ester, bexyl ester, octyl ester or dodecyl and the like.

Examples of the classes of compounds to be used in this composition administered through inhalation therapy include, but are not limited to (1) bronchodilators, such as metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, and salmeterol; (2) antiinflammatory steroids, such as BDP, dexamethasone, prednisolone, hydrocortisone, fluoromethasone, medrysone, fluticasone, triamcinolone, and flunisolide; (3) anticholinergics, such as atropine methyl nitrate, ipratropium bromide, (4) mast cell stabilizers,

including cromolyn sodium and nedocromil, (5) cardiovascular compounds, (6) oncology drugs for treatment of lung cancer such as, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide and altretamine, (7) antiviral drugs, including acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine and amantadine; (8) antibiotics including penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin; (9) antifungals, including amphotericin B and micozanole (10) cardiac drugs such as antihypertensives including apresoline, atenolol, captopril, verapamil, enalapril, antiarrhytmics including dopamine and dextroamphetamine; (11) antiparasitic drugs such as pentamidine; (12) antihistamines and immunotherapeutics including pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin; (13) hormones such as ACTH, insulin, gonadotropin; (14) tranquilizers, sedatives and analgesics such as dilaudid, demerol, oxymorphone, hydroxyzines; and (15) vaccines hemophilus influenza, pneumococcus, HIVs and respiratory syncitial virus, their salts or ester, alone or in combination.

The increased retention of the drug in the liposomes can be exploited in any type of delivery systems, such as inhalation, parenteral, intravenous or topical steroid administration and devices in solid, liquid, aerosol, nebulized, cream or spray form.

The liposomal composition of the invention can be prepared and delivered in a number of ways. For inhalation therapy, the delivery is achieved by (a) aerosolization of a dilute aqueous suspension by means of a pneumatic or ultrasonic nebulizer, (b) spraying from a self-contained atomizer using a propellant solvent with suspended, dried

liposomes in a powder, (c) spraying dried particles into the lungs with a propellant, or (d) delivering dried liposomes as a powder aerosol using a suitable device.

The improved retention of the drug in the liposomes can be advantageously exploited in intravenous or topical delivery systems in devices, intravenous infusions, injections, capsules, cremes, drops, lotions, ointments. It can be used for treatment of infection, inflammation, injury, or diseased conditions involving eye or skin wounds, rheumatoid arthritis, joint inflammation, allergic reactions, hormonal disturbances, asthma, emphysema, intestinal fibrosis, respiratory disease syndrome, cardiovascular disorders, infections, and other inflammatory conditions and allergic conditions.

The composition of the current invention shows a great stability, thus increasing a shelf-life of the drug for extended period of time for up to one year.

An added benefit to the liposome delivery system is that it can be used for combination therapy. For instance, in certain asthmatic conditions, a steroid is used for antiinflammation, or as antiallergenic agent while a bronchodilator is needed to relax the bronchial muscle and expand the bronchial air passages. Both can be incorporated in the liposomes for slow release. Antibiotics, antivirals, antiallergens, vitamins, nutrients, or any other water-soluble compound can be used when dual therapy is needed to counteract the immunosuppressive characteristics of steroids.

The following examples illustrate methods of preparing nonconventional liposomes suitable for formulation of steroid drugs and for use of these nonconventional liposomes for therapeutic purposes. These examples are in no way intended to limit the scope of the invention.

EXAMPLE I

Preparation of Conventional Liposomes By Thin Film Hydration

This example illustrates preparation and encapsulation efficiency of conventional phospholipid liposomes incorporating steroid beclomethasone dipropionate (BDP).

Liposomes were formed by modified thin film hydration method according to BBA, 691:227 (1982). Unlabeled BDP obtained from Sigma was spiked with ^{14}C-BDP. Labeled synthetic lipid dipalmitoylphosphatidyl choline ^{3}H-DPPC (from New England Nuclear) in trace amounts was used as a lipid marker in addition to lipid determination by inorganic phosphate analysis. Conventional liposomal formulations containing steroid and phospholipid in the ratios indicated in Table 1 were prepared as follows:

A. 1 mole % of BDP spiked with ^{14}C-BDP and 99 mole % of partially hydrogenated egg phosphatidylcholine spiked with ^{3}H-DPPC were combined in a round bottomed flask, and dissolved in 5 ml of chloroform. The solvent was removed by a rotary evaporator at room temperature and vacuum dried for one hour under a lyophilizer. The residual thin dry lipid film was hydrated with 3 ml of phosphate buffered saline of pH 7.4 by placing the round bottomed flask on a rotary evaporator without vacuum for one hour at 30^{o} and subsequently, under gentle shaking, on a mechanical shaker overnight at room temperature.

The MLV's formed were heterogeneous in size between about 0.05 to 20 microns, and a predominance of multilayered structures. These liposomes were extruded through a 0.4 or a 0.2 micron polycarbonate membrane by using a stainless steel extrusion cell (Lipex Biomembrane, Inc., Vancouver, British Columbia, Canada) to produce uniform homogeneous size distribution and to remove free drug crystals.

B. Using the procedure of Section A, 1 mole% of BDP, 96 mole% of egg phosphatidylglycerol and 3 mole % of egg phosphatidylcholine was formulated as formulation B.

C. Using the procedure of Section A, 10 mole % of BDP, 60 mole % of egg phosphatidylcholine and 30 mole % of cholesterol sulfate was formulated as formulation C.

Table I illustrates the encapsulation values and efficiency of various conventional and nonconventional liposome formulations.

Table I

	Formulation[5] Mole%	Drug/Lipid Ratio Initial[1]	Drug/Lipid Ratio Final[2]	Encap. Effic.
A.	EPC:EPG:BDP (96:3:1.3)	0.013	0.011	85%
B.	EPC:BDP (98:2)	0.020	0.015	75%
C.	EPC:BDP (95:5)	0.050	0.020	40%
D.	PHEPC:BDP (99:1)	0.010	0.008	80%
E.	PHEPC:BDP (99:1)	0.010	0.010	80%
F.	DLPC:DLPG:BDP[3] (96:3:1)	0.010	0.010	100%
G.	EPC:LEPC[4]:BDP (90:8:2)	0.020	0.019	95%
H.	EPC:$CHSO_4$:BDP (60:30:10)	0.100	0.012	12%
I.	$CHSO_4$:CH:BDP (53:37:9)	0.090	0.090	100%
J.	$CHSO_4$:CH:BDP (50:40:10)	0.100	0.100	100%
K.	$CHSO_4$:CH:BDP (55:40:5)	0.050	0.050	100%
L.	$CHSO_4$:CH:BDP (50:40:10)	0.100	0.100	100%

1. Amount formulated.
2. After formulation and removal of non liposome associated free drug.
3. DLPC and DLPG refer to dilauroyl phosphatides.
4. LEPC refers to lyso egg phosphatidylcholine.
5. All liposomes were formulated at 40 u mole/ml total lipid concentration.

Initial drug/lipid ratio refers to percent mole fraction of the drug used in the formulation. The final drug/lipid ratio means mole % fraction of drug in liposomes after formulation and removal of free drug not associated with liposomes. The encapsulation efficiency shows the amount of the steroidal drug which can be encapsulated in various nonconventional (I-L) or conventional (A-H) liposomes. As can be s n the conventional phospholipid containing liposomes can have rather high encapsulation

efficacy with respect to limited amount of drug used in the formulation. But final drug/lipid ratio shows that only 2 mole % of drug could be incorporated into these liposomes at total lipid concentration of 40 umole/ml.

Nonconventional liposome formulations prepared as described in Example III below, show high encapsulation efficiency at high drug concentration. The overall encapsulation of steroid in nonconventional liposomes was around 100% even when 10 mole % drug was used in the formulation with requirement for the amount of lipid approximately 10 times lower than for conventional liposomes.

Beclomethasone dipropionate phospholipid liposome formulations were tested for their release behavior in an in vitro and in vivo exchange with membrane systems as described in Examples V and VI.

EXAMPLE II

Preparation of Conventional Liposome Formulation by Solvent Injection Technique

This example describes the preparation of conventional liposomes using the procedure described in U.S. Patent 4,235,871,

A. A mixture of partially hydrogenated egg phosphatidylcholine (PHEPC IV-40, 1.98 mmol), and steroid (BDP, 0.02 mmol), in the mole ratio of 99:1 was spiked with radioactive label as in Example I.A. and dissolved in 100 ml of Freon 11 containing 1.0 ml of ethanol. Liposomal BDP dispersion was formed by slowly injecting the lipid/drug/freon solution into 50 ml of the phosphate buffered saline pH 7.4 under the following conditions: Injection rate: 1.25 ml/min; Vacuum: 400 mm Hg; Temperature: 20°C; Mixer rate: 1000 rpm. After the injection was completed, the vacuum level was adjusted to 150 mmHg for about 30 min to remove residual solvent. Liposomes thus formed were extruded through a 0.4 or a 0.2 micron polycarbonate membrane to produce uniform size liposome

distribution and to remove free drug crystals. Resulting liposomes were submitted to in vitro exchange assay described in Example VI.

B. Using the procedure of Section A, 1 mole% of BDP, 96 mole% of egg phosphatidylcholine and 3 mole% of egg phosphatidylglycerol was formulated as formulation B.

C. Using the procedure of Section A, 10 mole % of BDP, 60 mole % of egg phosphatidylcholine and 30 mole % of cholesterol sulfate was formulated as formulation C, substituting freon with solvent alcohol/freon or alcohol/chloroform (2:1).

EXAMPLE III

Preparation of Nonconventional Liposomes

This example illustrates the method for preparing the nonconventional cholesterol, cholesterol sulfate containing liposomal composition for sustained release of steroids.

^{14}C-BDP used as a marker in formulations was obtained by conversion of ^{14}C sodium propionate (1 mCi, Sp. Act. 56 mCi/mmol) to propionic anydride which was used to acylate nonlabeled beclomethasone in the presence of acylation catalyst dimethylaminopyridine. ^{3}H-cholesterol sulfate was synthesized according to a scaled-down and modified version of Mandel procedure described in Biochem. Zeit., 71:186 (1915).

A. Steroidal drug BDP (10 mole%) and lipids cholesterol sulfate (50 mole%) and cholesterol (40 mole%) in amounts (40 u mole/ml per liposomal formulation) were dissolved in 10 ml methanol:chloroform (2:1), added to a screw-cap test tube and dried under nitrogen. The procedure was repeated three times and the dried film was lyophilized for half an hour at room temperature. Depending on the liposomal volume needed, the residue was resuspended in about 2 to 5 ml of phosphate buffered saline (pH 7.4, mOsm - 295, originally preserved with sodium azide) and sonicated with a bath sonicator (Model G112SP1T, 600 volts, 80 KC, .05 Amps) for half an hour to prepar multilamellar vesicles

(MLVs). An aliquot of the sonicated, pre-extruded MLVs sample was saved and volume of preparation recorded for determination of baseline values. Liposomes were then extruded with a stainless steel Cullis high pressure extrusion cell one time through a 8.0 um Nucleopore polycarbonate membrane and two times through a 0.4 um Nucleopore polycarbonate membrane at $\leq$ 500 psi using the extrusion method described in U.S. Patent 4,737,323.

A post-extrusion sample was saved to determine the amount of drug or lipid lost in the sizing process. Post-extrusion volume was noted. Free drug, if any, was removed by repeated washing with phosphate buffered saline and centrifugation. Liposomes were centrifuged three times on the Beckman L8-70M Ultracentrifuge at temperature of 4°C, at 47,600 rpm, for 1 hour, using 50 Ti rotor. The supernatant was discarded and the pellet resuspended in a volume equal to the post-extrusion volume after each centrifugation. The cleaned sample obtained by resuspending the pellet after the third centrifugation was labeled as T_0 sample. This sample was saved to determine percent encapsulation.

All liposome formulations I-L (Table I) were prepared according to this procedure.

B. Using the procedure outlined above, dexamethasone, hydrocortisone, prednisolone, fluoromethasone, medrysone, and all other steroids are similarly formulated in nonconventional liposomes.

EXAMPLE IV

Encapsulation Efficiency and Stability

This example illustrates lipid compositions screened by varying the level of drug BDP, by determining the amount of the drug incorporated into the liposomes i.e. drug encapsulation, and by monitoring the stability of drug that remains associated with liposomes over time (Table I).

Multilamellar vesicles (MLVs) were formed containing ^{14}C BDP in phosphate buffered saline at pH 7.4 and extruded through a 0.4 micron polycarbonate membrane as described

above in Example I. The samples were washed and centrifuged several times to remove the free drug that is not associated with the liposomes according to Examples 1-3.

The vesicles were visually examined under a light microscope to detect the presence of drug crystals. No crystals were observed after encapsulation of steroidal drug BDP into nonconventional liposomes. Conventional liposomes had to be washed to remove the excess of the drug before they were microscopically clear of crystals. In addition BDP incorporation was low.

The level of incorporation of the drug in the liposomes was determined based on radioactive counts and expressed as encapsulation efficiency as shown in Table I.

The stability of the incorporated steroidal drug in the liposomes was followed for several days to several months. For these stability studies, liposome samples obtained above were further diluted with PBS at pH 7.4 (1:5 v/v) and incubated at ambient temperature. Time aliquots were withdrawn and pelleted by centrifugation (19,000 rpm, 4°C, 30 min). The supernatant and pellets were monitored for the presence of lipid and drug. After the liposome preparations were diluted, the amount of drug remaining in the liposomes after three days to three months was determined to assess the stability of the incorporation. Very little, if any, of the steroid leaked out of the nonconventional liposomes after three days indicating that the incorporation was very stable at ambient temperature.

Nonconventional liposomes also showed no crystals after three months of storage at 4°C by light microscopy. Conventional liposomes, although appearing stable for 3 days at ambient temperature in buffer solutions, lost readily their drug content during the longer period of storage and/or in the presence of an acceptor membrane. Conventional liposomes such as A-G (Table I) even though they showed no crystals after 3 m nths at 4°C, readily lost

the drug content both in vitro in the presence of a membrane reservoir (Table II) and in vivo.

EXAMPLE V

In Vitro Membrane Exchange Assay

This example illustrates the sustained release from the nonconventional liposome formulations prepared according to the current invention.

An in vitro membrane exchange assay for measuring the release of drug from liposomes was established for screening of all formulations before conducting bioavailability studies.

BDP, as a steroid poorly soluble in water, and is primarily entrapped in the lipid bilayer rather than in the aqueous core of liposomes. Thus, very little of the drug can be released into a surrounding aqueous environment unless a huge volume of buffer is used based on partitioning characteristics of the drug. Since BDP has good solubility in phospholipid membranes, liposomal BDP may be rapidly exchanged from the bilayer of liposomes to surrounding cell membranes in the lung. To mimic the cell membranes in the lung, in vitro system was set up using small unilamelar vesicles (SUVs) .

An aliquot of conventional liposome formulation of BDP (EPC:EPG:^{14}C-BDP/96:3:1) prepared in Example I was mixed with an equal volume (50:50) adjusted to the same total molar lipid amounts of non-drug containing empty EPC SUVs' prepared according to procedure of Example I with EPC as the only lipid. Both, drug containing and empty liposomes (MLVs or SUVs), were mixed and incubated at 37°. Samples were taken at 0, 0.5, 1, 2 and 4 hours. Samples were centrifuged at 4°C for one hour to separate the drug-containing liposomes (pellet) and the empty SUVs liposomes (supernatant). Pellets and supernatants were analyzed for radioactivity. Approximately half of all radioactivity was found in the supernatant for all time p ints, indicating that the drug was rapidly transferred from the drug-containing

conventional liposomes to the empty SUVs until an equilibrium was reached between the two types of membranes.

This experiment was done with formulations EPC:EPG:BDP (96:3:1) and PHEPC:BDP (99:1). Both these formulations (Table II, A and B) had a high percentage of the drug exchanged from drug containing liposomes to empty SUVs, namely 89% for EPC:EPG:BDP and 85% for PHEPC:BDP.

Because of the rapid transfer of BDP into the SUVs, only the initial time point was used in subsequent studies. The ratio of drug-containing to non-drug containing liposomes was varied from 1:1 to 1:25. Results showed that at a ratio of 1:5 (donor/acceptor liposomes) bulk of the drug was rapidly exchanged to acceptor membranes.

The same method was then used to measure the amount of drug released from three nonconventional liposome formulations of BDP (Table II, D-E) and one conventional liposome formulation containing cholesterol sulphate (Table II C). From three nonconventional liposome formulations containing cholesterol sulfate, CH:$CHSO_4$:BDP 40:50:10; 40:55:5 and 37:53:9 mole %, none of the drug was released. Conventional phospholipid liposomes containing cholesterol sulfate (EPC:$CHSO_4$:BDP/60:30:10) which were not able to incorporate more than 1.2 mole % of the drug, released 9% of the incorporated drug to the acceptor SUVs. These vesicles also behaved like conventional liposomes types in animal models (Example VI).

From the formulations containing combination of cholesterol sulphate and cholesterol with steroid, none of the drug was released into the supernatant and thus no drug was transferred between drug containing liposomes and empty liposomes.

Table II

Results of In Vitro Membrane Exchange Assays

	Formulation	Percent of Drug Transferred
A.	EPC:EPG:BDP (96:3:1)	89%
B.	PHEPC:BDP (99:1)	85%
C.	EPC:$CHSO_4$:BDP (60:30:10)	9%
D.	$CHSO_4$:CH:BDP(50:40:10)	0%
E.	$CHSO_4$:CH:BDP(55:40:5)	0%
F.	$CHSO_4$:CH:BDP(53:37:9)	0%

EXAMPLE VI

In Vivo Studies

This example illustrates in vivo studies with nonconventional liposomes and their potential for sustained release.

All studies were performed in male Sprague-Dawley rats weighing 250 to 450 g. After fasting for 16 hours, animals were anesthetized by i.m. injection of ketamine (25 mg/kg), xylazine (5 mg/kg) and acepromazine (0.5 mg/ml). During the procedure the animal's body temperature was maintained with a 37°C heating pad. Additional anesthetic was administered as required, using half the original dose. A midline incision was made in the neck and the right jugular vein and left carotid artery were cannulated with short lengths of polyethylene tubing to which a 23 ga Luer stub adapter (Clay Adams #7565) and plastic 3-way stopcock (Argyle #173518) were attached. Blood samples (0.5 - 4 ml) were removed from the carotid arterial cannula after first flushing with fresh blood to clear the line. Blood volume removed was replaced with an equal volume of 5% dextrose solution containing 50 U/ml of heparin via the jugular cannula. For intravenous (i.v.) injection studies, drug was injected into the venous cannula with a 500 ul glass syringe via a 22 ga needle and injection cap and flushed with 0.5 to 1.0 ml dextrose solution.

For intra-tracheal (i.t.) instillation of BDP formulations, (Table III) the trachea was cannulated with a

4 cm long section of Teflon tubing (1.2 mm I.D.), inserted at the level of the fifth tracheal ring below the thyroid cartilage and tied in place with a suture. Excess fluid in the trachea was aspirated through tubing attached to a syringe. A 0.5 ml glass syringe with a blunt needle and short length of polyethylene tubing attached was used to administer the formulations. The tubing was inserted to the level of the bronchial bifurcation and the dose (100 to 400 ul) rapidly administered during an inhalation. Animals were supported head up on a tilted dorsal support (approximately 70°) during the instillation process.

Blood samples were removed at four time points during the study from each rat, centrifuged, and the serum was removed and stored frozen (-20°C) until assayed. Lung tissue samples were collected by rapidly excising the lungs after the final blood sample and immediately homogenizing in ice-cold acetonitrile (10.0 ml). The homogenate was briefly centrifuged and measured aliquots of the supernate removed to Teflon-stoppered glass tubes which were stored at -20°C or below until assayed.

Analysis of plasma and lung tissue samples for ^{14}C-BDP was carried out by liquid scintillation counting. The actual dose administered in each study was determined by measurement of duplicate dose control samples of the formulation which were delivered by the same apparatus used in dosing the animals.

Table III

Intratracheal Instillation to Sprague-Dawley Rats.

Liposome Formulation (mole%)	Dose BDP (mg/kg)
$CHSO_4$:EPC:BDP 32.9:65.8:1.3*	0.007
$CHSO_4$:CH:BDP 53:37:9	0.187
$CHSO_4$:CH:BDP 50:40:10	0.260
$CHSO_4$:CH:BDP 55:40:5	0.260
$CHSO_4$:CH:BDP 50:40:10	0.035

*This formulation was prepared at 60:30:10 (molar ratio). Since BDP was incorporated only to the extent of 1.2 mole % of original amount, the ratios were adjusted accordingly.

Each of the liposomal BDP formulations shown in Table III was administered to a group of 12-18 rats as described above. Groups of 3-6 rats were sacrificed at each of three time points during each study and the amount of radiolabeled BDP remaining in the lungs was measured by liquid scintillation counting. In some studies, the plasma concentration of radiolabel was also measured over the course of the experiment.

The pharmacokinetic parameters of free BDP were determined following intravenous administration of ^{14}C-BDP (0.008 mg/kg in 50% aqueous ethanol) to a group of 12 rats. Plasma and lung levels of radiolabel were measured as previously described. The decrease in plasma concentration versus time following free drug administration was biphasic (Figure 4). These data were subjected to analysis by a non-linear least squares curve fitting program (RSTRIP, MicroMath, Salt Lake City, UT) and the resulting exponential slopes and intercepts interpreted according to a two

compartment open pharmacokinetic model.

The plasma kinetics observed following the i.t. instillation of ^{14}C-BDP (0.007 mg/kg) incorporated into EPC/cholesterol sulfate liposomes were virtually identical to those observed following the i.v. administration of a similar dose of free drug (Figure 4). The amount of radiolabel remaining in the lungs after 35 minutes was only 1% of the total administered dose for this formulation (Figure 6). These data indicate that BDP is rapidly and completely absorbed from the lungs after instillation of this formulation.

The absorption kinetics of nonconventional liposomal formulations were found to differ significantly from those of free drug and formulation containing EPC and cholesterol sulfate (Figure 6). Significant amounts of radiolabel were detected in the lungs over the course of the study for each of the four cholesterol/cholesterol sulfate formulation studied. In contrast, 98.8% of the ^{14}C-BDP in EPC/CHS liposomes had left the lungs 30 minutes after administration and 99.7% of free ^{14}C-BDP was absorbed in the same time period. These results demonstrate that sustained in vivo release of liposome incorporated BDP had been achieved.

Corresponding plasma concentration versus time data were obtained for one of the sustained release formulations (Figure 7). The plasma concentration versus time curve observed after administration of ^{14}C-BDP (0.187 mg/kg) in a cholesterol/cholesterol sulfate liposome formulation was strikingly different from that of free drug, remaining nearly flat over the two hour duration of the study (Figure 6). Since lung data indicated that 27% of the administered dose was free or rapidly released drug, the plasma concentration curve for this study reflects the sum of concentrations due to "free" and "encapsulated" drug. The concentration time curve for "free" drug was estimated by assuming 27% of the dose was immediately absorbed and

followed the kinetics observed for i.v. administration of free BDP. This curve was subtracted from the experimentally observed data to give an estimate of the plasma concentration due to liposomal (sustained-release) BDP (Figure 7).

The present study shows that the lipophilic steroid beclomethasone dipropionate can be successfully incorporated into a nonconventional liposomal formulation that provides sustained in vivo release of the drug following intratracheal instillation.

Table IV illustrates the in vitro and the in vivo exchange of conventional and nonconventional liposomes.

Table IV

Formulation	In Vitro Exchange	In Vivo Exchange
EPC:BDP 98:2	+	+
EPC:EPG:BDP 96:3:1	-	-
EPC:$CHSO_4$:BDP 50:40:10	-	-
$CHSO_4$:CH:BDP 53:37:9	-	-
$CHSO_4$:CH:BDP 55:40:5		

Example VII

Preformulation Studies

This example determines the localization of the steroid in the liposomal structure and illustrates the steroid's water insolubility. Beclomethasone dipropionate is a lipophilic drug. The solubility of the drug in different solvents is listed below in Table V:

Table V

Solvent	Solubility
Ethyl Alcohol	16.7 mg/cc
Chloroform	125 mg/cc
Acetone	Highly soluble
Water	54.4 ug/cc*

* determined using radiolabeled material.

The partition coefficient for beclomethasone dipropionate between octanol and phosphate buffer saline was determined at pH 7.4. Nearly all (95%) of the BDP was associated with the octanol. This indicates that the drug will most likely reside in the membrane core of the bilayer.

EXAMPLE VIII

Intrathecal Administration of Liposomal Steroids

This example illustrates the intrathecal administration of the steroids formulated in nonconventional liposomes. The treatment is useful for boosting the effect of antibiotic or other treatments in severe sepses, blood poisoning, meningitis, brain inflammations and infections or other conditions when the immediate and prolonged administration of the steroid is indicated.

Male Sprague Dawley rats, 3 to 5 months old, weighing between 360-460 grams are anesthetized with sodium pentobarbital (45 mg/kg;i.p.) and mounted in a conventional stereotaxic frame. A midline incision is made to expose the dorsal surface of the skull. A small hole (1 mm) is drilled through the calvarium at a point 0.4 mm rostral and 1.8 mm lateral to the bregma. The dura is torn with a sharp needle and a 30 gauge blunt needle tip is lowered into the brain to a point 4.2 mm below the skull surface into a lateral ventricle.

The rats are divided into one experimental and one control group. The experimental group is injected with 50 ul of liposomal cortisone composition of 50 mole % of cholesterol sulfate, 40 m le % of cholesterol and 10 mole % of cortisone (spiked with radioactive cortisone) prepared

according to procedure of Example III. The control group is injected with 50 ul of the free cortisone 20 mg/ml suspended in 0.9% NaCl. Injection is done over 25 minutes using a syringe infusion pump. At the end of the injection, the needle is removed and the skin defect is closed with a surgical staple.

At appropriate time points, three rats per time point from each group are sacrificed with an overdose of sodium pentobarbital (100 mg/kg i.p.), the blood specimens are obtained from cardiac puncture and the animals are allowed to exanquinate completely. The skin overlying the calvarium is removed and the calvarium is carefully removed. 20 ul of cerebrospinal fluid is obtained by carefully making the small tear in the underlying dura in the frontal area and pipetting out the fluid. The cerebrospinal fluid is diluted with 100 ul of 0.9% NaCl solution, centrifuged in a microfuge for one minute, and the supernatant containing free drug is separated from the pellet containing liposomes before storage at 20°C. The brain in the cranial cavity is lifted out with a spatula and the cranial cavity is washed out thoroughly with a 0.9% NaCl solution to collect all drug remaining in the cranial compartment. The spinal cord is extruded forward into the cranial vault by inserting in the rostral direction a 19 gauge hypodermic needle in the low lumbar spinal canal at a point 2.5 cm rostral to the origin of the tail and then pushing 0.9% NaCl solution into the canal at high pressure. The empty spinal canal is then washed thoroughly with 0.9% NaCl solution to collect all the drug in the spinal canal. The brain compartment specimen is collected separately from the spinal specimens. The specimens are homogenized on ice with distilled water using a Dounce manual tissue grinder, sonicated to disrupt intact liposomes and filtered through the ultrafiltration membrane (YMT membrane). The ultrafiltrates are analyzed with HPLC.

The amount of the drug is measured in cranial and spinal compartments and in cerebral fluid.

In all cases, the liposomal steroid remained in the cranial or spinal compartments for as long as 24 days while the free drug almost completely disappears within the first 2.8 hours. In the cerebrospinal fluid the free drug appears immediately in the high concentration and is quickly eliminated from the cerebrospinal fluid within the 2.8 hours. The liposomal cortisone, on the other hand remains in the cerebrospinal fluid for as long as 16 days.

EXAMPLE IX

Intraarticular Injection of Nonconventional Liposome Steroidal Composition

This example illustrates the use of the nonconventional steroidal liposomes for treatment of arthritis, rheumatoid arthritis, tendonitis and other inflammatory diseases of the joints by injection of liposomal steroid into joints.

New Zealand rabbits of 2.5 to 3.5 kg are shaved around the joints of both hind legs. Between 8 and 9 a.m. the right joints received an intra-articular injection of 0.5 ml of freshly prepared nonconventional liposomes with encapsulated hydrocortisone (3 mg) the left joints are injected with 0.5 ml physiological saline as control. Blood samples are collected from the ear veins at timed intervals. The rabbits are kept in metabolism cages. The rabbits are anesthetized 24 or 48 hours after the i.a. injection and the joints rinsed with 2 ml of physiological saline ("synovial fluid"). Then they receive 1 ml of Disulphine Blue™ 6.2 per cent s.c., between the toes of both hind paws to stain the lymph nodes. At death total synovectomy of both joints is carried out, the heads of the femur, fibula and tibia and the popliteal lymph nodes excised. The tissues were grouped as follows: (1) menisci, tendons, ligaments and cartilage scraped off from femur, fibula, tibia, patella and fabellas were put together and named as "menisci etc.", (2) "synovium", (3) "patella and fab llas", (4) "femur, fibula and tibia-heads", (5) "bone marrow" which was scraped out

from femur, fibula and tibia, (6) popliteal "lymph nodes". Menisci etc., synovium and bone marrow are digested with Packard Soluene 350, the bones with concentrated $HClO_4/H_2O_2$(1:2 v/v). Aliquots of whole blood, plasma, synovial fluid and after centrifugation and the digested materials are subjected to liquid scintillation counting. In the fresh synovia the number of cells is counted and cell differentiation is carried out. Urine and faeces are collected daily, aliquots of urine are subjected to liquid scintillation counting directly, aliquots of faeces after digest with Packard Soluene 350.

Nonconventional preparations give indistinguishably low plasma levels of radiolabel for 48 hour post i.a. injection. The levels range from 0.001 to 0.3 per cent of the dose in the whole rabbit blood.

The liposomes have shown the long term 150 hours retention of radiolabel in synovial fluid and therefore in the whole joint.

EXAMPLE X

Treatment of Arthritis by Intraarticular Administration of Steroid in Nonconventional Liposomes

This example illustrates the effect of nonconventional liposome steroidal formulation in treatment of arthritis.

Experimental Arthritis

Male and female Old English rabbits (1.8-2.4 kg) are used. Before induction of the arthritis, hair is removed from both knee joints by the use of a commercial depilatory cream. A bilateral arthritis is induced by the intraarticular injection of a preformed insoluble complex of poly-D-lysine and hyaluronic acid into both knee joints (Shaw et al., 1979).

An acute inflammatory flare, superimposed on an underlying chronic arthritis, is induced by giving a second injection of the polylysine-hyaluronate complex 28 days after the first injection.

Measurement of Inflammation

Radiometric measurement of joint temperature was carried out with a Heimann KT41 radiation thermometer (Phillips and Phage Thomas, 1979). The radiation from an area of skin 0.6 cm in diameter situated on the anterolateral side of the knee over the joint space is measured. The diameter of the knee is measured in the coronal plane of the articular space with a calibrated spring-loaded Baty micrometer.

Treatment of Experimental Arthritis

Liposomes (0.5 ml) containing the cortisol (3 mg) encapsulated in nonconventional liposomes prepared according to Example III and conventional liposomes prepared according to Example I, are injected into one knee joint 4 days after the induction of the arthritis. The temperatures and diameters of injected and contralateral joints is monitored for 3-5 days after treatment. In the treatment of an acute inflammatory "flare" superimposed on a chronic arthritis, liposomes are injected 4 days after induction of the acute episode.

The effect of treatment of an experimental arthritis in rabbits with nonconventional liposomes containing cortisol is investigated after the arthritis had developed for 4 days. It had previously been demonstrated that maximum anti-inflammatory activity was observed when liposomes were injected at this time, decreased activity was observed when treatment was started at 8 and 15 days after induction.

Antiinflammatory activity of steroid encapsulated in nonconventional liposome showed a significant and sustained reduction in both joint temperature and diameter when compared to steroid encapsulated in conventional liposomes.

EXAMPLE XI

Intravenous Administration of Nonconventional Liposome Steroid Composition

This example illustrates the use of the nonconventional steroid liposomes for intravenous treatment of various diseases requiring steroidal treatment.

The nonconventional steroidal liposome composition prepared according to Example III was administered intravenously and the ability of mouse organs to bind and/or take up the radiolabel was studied. Liposome formulation was concentrated, if necessary, by adding one volume of 50% (w/v) sucrose in phosphate-buffered saline to 3 volumes of liposome suspension, centrifuging at 12 800 x g for 10 minutes removing the concentrated upper liposome layer and diluting to required volume with phosphate-buffered saline.

All experiments were carried out using groups of male ICR mice weighing approximately 25 grams (the weight of any individual mouse was not more than 10% different from the group mean weight). Doses of nonconventional liposomes containing beclomethosome dipropionate spiked according to Example III were administered to a mouse via a tail vein. At the end of the experimental period mice were lightly anesthesized with diethyl ether and a 1 ml blood sample was rapidly removed from the jugular vein with a heparinized syringe. Livers and spleens were subsequently removed, weighed and set aside for analysis together with the remaining carcass. An attempt was made to remove and discard the bladder and its content from each carcass prior to storage. Three types of studies were performed as follows.

Three groups of three experimental mice were each given 0.2 ml phosphate-buffered saline containing free radioactive spike beclomethasone intravenously via a tail vein. Mice receiving imperfect injections were discarded. Groups of three mice were killed at 1, 5 and 24 hours after injection and their organs sampled as described above in

order to determine the clearance of free beclomethasone.

All organ and carcass samples were stored at -20° prior to analysis. Aliquots of liver (approximately 0.2 grams), spleen (whole organ), whole blood (approximately 0.8 ml) and of the dose (0.05 ml) were transferred to pre-weighed combustion cups, re-weighed and then allowed to air-dry. The remaining carcass was placed in a container together with 75 ml of water and homogenized using a Williams polytron wet milling device (Brunwell Scientific, Rochester, NY). Samples of the resulting slurry (approximately 1.5 grams) were weighed into combustion cups and allowed to air-dry for at least 24 hours. Dried samples were analyzed for total ^{14}C radioactivity by scintillation counting following combustion in a Packard Sample Oxidizer, Combustions were carried out in series, with the inclusion of appropriate blanks. A duplicate sample from every third animal was analyzed to monitor the combustion reproducibility. Values of sample cpm were corrected for variations in quench by use of a quench curve and averaged over the three experimental animals in a group. Total radioactivity in liver, spleen, carcass and 1.0 ml blood was calculated and converted to percent of encapsulated dose. When calculating carcass values an allowance for blood volume remaining after sampling was made, assuming the total blood volume of a mouse equals 7.3% body weight. Total radioactivity in vivo was estimated by summing values for liver, spleen, total blood volume and carcass.

Plasma concentration of free and encapsulated beclomethasone in nonconventional liposomes was determined. The results are shown in Figure 7. Free beclomethasone disappears rapidly from the plasma, while the beclomethasone encapsulated in nonconventional liposomes remains circulating in the plasma as liposome plasma reservoir from which the amount of steroid is slowly released to the circulation.

EXAMPLE XII

Intraperitoneal Administration of Nonconventional Liposome Steroid Composition

This example illustrates the use of nonconventional steroid liposomes for intraperitoneal treatment of internal inflammatory diseases.

Male Sprague-Dawley rats weighing 250-300 grams are used. Each rat is given an oral dose of soybean oil (4.0) mL/kg) and 1 hour later anesthetized with an injection of urethane (1.2 g/kg sc). A polyethylene cannula (PE 10, Clay Adams) is inserted into the thoracic duct proximal to the juglosubclavian junction according to the method of Saldeen and Linder, Acta. Path., 49:433(1960). Another cannula (PE-50) is put into the left femoral artery, and a third is used to cannulate the urinary bladder. The anesthesia is maintained for the duration of the study. The rat is placed on a plate and kept at 37°C in a supine position. Fluid balance is maintained with a 4 mL/h/kg infusion of saline containing 2.5 U/mL of herparin via the arterial cannula. The test liposome steroid formulation according to Example III or free drug suspension (5 mM in phosphate-buffered saline, 2 mL/kg) is administered intraperitoneally 30 minutes after surgery. Lymph and urine are collected continuously. Blood is sampled periodically over the 5-h study period. At the end of the study, rats are sacrificed and the peritoneal cavity is rinsed with at least 60 mL of saline and 20 mL of 1% Triton X-100 to recover unabsorbed liposomes and marker. Several lymph nodes in the portal, and superior mesenteric) and those around the thymus (left and right mediastinal, and parathymic) are excised for assay. The molecular weight cut-off of the peritoneal-vascular permeability barrier is determined using sucrose, inulin, and fluorescein isothiocyanate (FITC) dextrans of molecular weights 20,000, 70,000, and 150,000; these are dosed intraperit neally as phosphate-buffered saline solutions (sucrose and inulin at 5 mM; FITC dextrans at 20

mg/mL with 2 mL/kg injected). Steroid incorporated in this nonconventional liposome was retained in the peritoneal cavity for significantly longer periods than free drug.

EXAMPLE XIII

Topical Application of Nonconventional Liposome Steroidal Composition

This example illustrates the use of the nonconventional steroidal liposomes for topical treatments.

The effect of topical application of the steroid fluocinolone, both encapsulating in liposomes and as a free drug dissolved in acetone, has been evaluated using the female hamster flank organ as a model system.

The nonconventional steroid liposome radiolabeled composition according to Example III was used.

The hamster flank organ test was carried out according to the method described in Endocrinology, 92:1216-1222 (1983).

The female hamsters are separated into six groups of five animals and treated according to the following scheme.

Group I: 4 ug fluocinolone (dissolved in acetone);

Group II: 20 ug of free fluocinolone (dissolved in acetone);

Group III: 40 ug fluocinolone (dissolved in acetone);

Group IV: 4 ug fluocinolone (encapsulated in nonconventional liposomes);

Group V: 20 ug fluocinolone (encapsulated in nonconventional liposomes);

Group VI: 40 ug fluocinolone (encapsulated in liposomes);

Group VII: 40 ug fluocinolone (encapsulated in liposomes).

The animals are treated once daily (five days a week). After 28 days of treatment the flank organs are excised for morphometrical and histochemical examination according to the method of Go s et al., Arch. Derm. Res., 273:333-341 (1982). Systemic absorption of free

fluocinolone was significant from the acetone solution, but negligible from the liposome system.

EXAMPLE XIV

Preparation of Mixed Micelles Containing Beclomethasone Dipropionate

This example illustrates preparation of mixed micelles containing steroid beclomethasone dipropionate.

3g of Tauranol WS (60 mg/ml) is dissolved in 15 ml of deionized water and 20 mg of beclomethasone dipropionate (0.4 mg/ml) and 450 mg of sodium chloride (9 mg/ml) are added. Deionized water is added so that the volume is made up to 50 ml. The mixture is slowly stirred at room temperature overnight, then the solution is filtered through 0.2 μ filter. Filter is discarded and amount of drug, pH and osmolality of the filtrate is determined. The recovery of drug in this composition is shown as C in Table VI.

The filtrate is then poured into nebulizer vessel and nebulized to generate microaerosol particles according to Example XV.

The same procedure is used but the initial amount of Tauranol is A. 750 mg (15 mg/ml) or B. 1.5 g (30 mg/ml), with 450 mg (9 mg/ml) of sodium chloride. The amount of drug is the same and the volume is made up to 50 ml.

The results of drug recovery is illustrated in Table VI.

Table VI

Composition of Mixed Micelles of BDP and Tauranol WS

Ingredients	Initial Amounts mg/ml			Recovered Amounts mg/ml		
	A	B	C	A	B	C
Beclomethasone Dipropionate	0.4	0.4	0.4	0.142	0.258	0.386
Tauranol WS	15	30	60	N.D.	N.D.	N.D.
NaCl	9	9	9.1	N.D.	N.D.	N.D.
pH	-	-	-	7.4	7.62	7.74
Osmolality/mOsm/kg				295	312	288
Deionized Distilled Water q.s. to 1 ml						

N.D. means not determined

As can be seen the recovered amount of beclomethasone depends on amount of surfactant detergent used and the highest recovered amount was around 98% when the 60 mg of Tauranol per ml of micelle solution was used.

Using the procedure described above, micelle solution of BDP and Tween 20 was prepared in amounts and with recovery amount shown in Table VII.

Table VII

Composition of Mixed Micelles of BDP and Tween®-20

Ingredients	Initial Amounts mg/ml	Recovered Amounts mg/ml
Polyoxyethylene monolaurate (Tween® 20)	60	N.D.
BDP	0.4	0.059
pH	-	4.25
Osmolality	-	321 mOsm/kg
NaCl	9	N.D.
Deionized Distilled Water q.s. to		1 ml

As can be seen the drug recovery was only approximately 15% with the same highest amount of surfactant (60 mg/ml).

Using the above procedure, micelle solution of BDP and poloxamer was prepared in amount as shown in Table VIII.

Table VIII

Composition of Mixed Micelles of BDP and Pluronic® F68 Prill (A) and Pluronic® F68 (B)

Ingredients	A mg/ml	B mg/ml
Pluronic® F68 Prill	60	
Pluronic® F68	-	60
BDP (Nominal)	0.4 mg/ml	0.4
BDP (assayed)	none	none

The results summarized in Tables VI-VIII show that not only quantity of the surfactant but also the type of surfactant is important for the amount of drug recovery in micelles. Among these three investigated, surfactant Tauranol in amount 60 mg/ml proved to be the best in terms of drug solubilization.

Micelle solutions are used for treatment of interstitial lung diseases by aerosolizing micelle solution and administering it by inhalation to a patient.

EXAMPLE XV

In Vitro Testing of Aerosolized Liposome Steroidal Formulation

This example illustrates in vitro testing of aerosolized liposome BDP formulation.

4 ml of presized (0.02μ) nonphospholipid liposome (or adequate volumes of the liposome formulation to provide known amounts of drug diluted to a total volume of 4 ml with sterile saline or suitable diluent) obtained in Example 4 or mixed micelles of Example 8 containing BDP were placed in single-use Mallinckrot Ultravent nebulizer and the compressor pump was attached according to Figure 10. The

compressed air generated by the compressor is introduced into the nebulizer and agitates the liposomal or micellar solution into mist of aerosolized particle droplets. These droplets enter via the valve into Andersen cascade impactor. The flow velocity of air through the impactor is adjusted to 28 L/minute. The aerosol mist is separated into preseparator chamber and into segments 0-7. The preseparator, and each stage of segments 0-7 are separated from each other by stages with decreasing sizes of pores. The aerosolized particles are then forced by the compressor to enter the impactor and are selectively deposited on the glass slides place on the stages when their sizes are bigger than the pores of that stage or pass through the to the next stage until they are deposited on the glass slide. The sizes of stages corresponding to various segments of the lungs are shown in Figure 11.

After enough amount of liposomes is aerosolized, for analytical purposes (time corresponding to human breathing time in clinical trials) glass slides are removed and the content of BDP is determined spectrophotometrically after solubilization.

Experimental protocol is as follows:

Equipment (Andersen Cascade impactor) is set-up as shown in Figure 10 after ascertaining that all sections of the equipment are clean. A blank trial is run with 4 ml of deionized water placed in the nebulizer to determine how long it takes to nebulize 1 ml. The flow meter is adjusted to allow the air flow rate of 28 l/min. 4 ml of liposome or micelle suspension (or diluted formulations in appropriate cases) is introduced into the nebulizer and nebulized to deliver approximately 1 ml of the material. Y-side arm, throat including mouthpiece, and all glass slides from stages of the impactor and submicronic aerosol filter are disconnected and removed. All of these sections where aer sol droplets have settled were rinsed separately with 5 ml of methanol/saline (0.9%) 4:1;v:v and the absorption

spectrum of BDP in methanol/saline were scanned and read against standard BDP curve.

The BDP concentrations were determined spectrophotometrically for the nebulizer solutions before and after aerosolization and on the aerosol output deposited in the stages and Y-side arm. The amounts of drug (BDP) in each location was calculated and the material balance was verified as follows:

$D_I = C_I \cdot V_I \quad D_F = C_F \cdot V_F \quad D_D = D_I \cdot D_F$

$D_R = D_Y + D_{S1-8} + D_{TH}$

D_I = Drug initially introduced into the nebulizer in μg or mg.

C_I = Initial drug concentration in nebulizer

V_I = Initial volume of solution in nebulizer

D_F = Drug present in the nebulizer residue

C_F = Final drug concentration in nebulizer residue

D_D = Total delivered drug

V_F = Final volume of solution in nebulizer

D_R = Total recovered drug

D_Y = Drug present in aerosol droplets deposited in the Y shaped side arm

DTh = Drug deposited on throat piece

D_{S1-8} = Drug deposited on the seven stages and the fine or submicronic aerosol particle filter (S_8)

D_F could be very high, implying that only water was aerosolized preferentially and "crystalline" drug remained in residue. This may very well be the case with BECOTIDE®.

Percent recovery and aerosolization rate was calculated as follows:

$$\% \text{ recovery} = \frac{D_R \times 100}{DD} \quad \text{Average aerosolization rate} = \frac{V_I - V_f}{t}$$

Phospholipid concentrations are determined similarly to verify that there is a comparable material balance with lipid as well.

The drug/lipid ratio in the initial and final solutions in the nebulizer with liposomal formulations is checked. Ratio should remain constant. Any deviation points to drug

crystallization and therefore unavailability for aerosolization.

Liposome particle size of the nebulization solutions are measured before and after aerosolization experiments using the NiComp laser particle sizer. Liposome particle sizes in the collected aerosol are also measured.

Typical results obtained with Cascade Impactor Analysis of commercial Becotide® formulation and the liposomal BDP formulation are summarized in Table XI. A graphic plot of this data by standard methods gives the mass median aerodynamic diameter (MMAD) of the aerosol droplets (Figure 12, 13). Drug/phospholipid deposition on stages and throat is used to extrapolate how much of the nebulizer output will reach "alveoli" assuming that aerosol particles of MMAD 0.02-2.1 μ can be deposited in deep lung. As seen in Figures 12 and 13, the Becotide suspension generates larger particles and more significantly only 3.2% of the aerosolized dose reaches stages 5, 6, 7 (Table IX) corresponding to alveolar region. On the other hand, liposomal aerosol droplets have very small size (MMAD 0.4 μ) and deliver 28% of aerosolized dose in stages 5, 6, 7 (Table IX) corresponding to alveolar region. Results are summarized in Table IX.

Table IX

Andersen Cascade Impactor Analysis
Of Nebulized Becotide And BDP Liposomal Formulation

Volume nebulized 4 ml

HPLC Values:

FRACTIONS	BDP (μg) Becotide®	Liposomal BDP
Total	194.4	979
Residue	116.7	604
y Joint	18.5	10.6
Throat	1.7	4.54
Collar	13.2	1.90
Stage 0	0.4	8.95
Stage 1	0	6.27
Stage 2	0.3	9.87
Stage 3	1.2	9.5
Stage 4	1.2	16.6
Stage 5	4.0	68.7
Stage 6	1.0	80.0
Stage 7	0	11.7
Filter	0	1.77
Recovered Drug Total:	157.3	939.7
Percent Recovery:	81	96
Alveolar Deposition:	3.2%	28.5%

EXAMPLE XVI

Steroid Therapy in Patients Suffering from Lung Disease

This example illustrates the clinical protocol and results of treatment of patients suffering from ILD.

Very recently, several studies have implicated T lymphocytes of the inflamed lung as the source of the pathology in their release of interleukin (IL2) and T cell growth fact
or and their ability to continuously proliferate making lung an immune organ (Spencer, H. Pathology of the Lung, (1985).

New Engl. J. Med., 308 793 (1983), Rev. Respir. Dis., 128:634 (1983)). Active pulmonary sarcoidosis therefore, simulates a relevant model to evaluate, in humans, the in vitro findings that corticosteroids can suppress the level of the lymphokine IL2 and T lymphocyte proliferation. Using bronchoalveolar lavage to sample activated T lymphocyte population, it is possible to monitor the effects of corsticosteroid formulations of the present invention in suppressing IL-2 levels (either by protein monitor using antibody or at genetic level using mRNA probes) and their ability to reduce cell proliferation in cell culture. The following experimental protocol was designed to monitor the disease course in patients with control population of normal volunteers.

Bronchoalveolar Lavage (BAL)

Prior informed consent was obtained from all individuals entering the study. Each subject was premedicated with 0.6 mg atropine sulfate i.m., 8 mg morphine sulfate and subsequently received aerosolized xylocaine (4%) and 2 puffs of Alupent®. Bronchoscopy was accomplished with a flexible fiberoptic bronchoscope (BR-4 B/2; Olympus Corp., New Hyde Park, N.Y.) which was wedged into a subsegmental bronchus prior to BAL. Lavage was performed by an injection of warm (36°C) saline in five 20 ml aliquots into subsegmental bronchi. Suction was applied immediately and the fluid was collected in a sterile trap. Generally volume recovered ranges from 55-70 ml. The lavage fluid was passed through 2 layers of sterile gauze and cells were pelleted at 250 g for 5 minutes and washed twice in RPM 1640 (M.A. Bioproducts, Walkersville, M.D.) and then resuspended to a concentration of 10^7 cells/ml before use.

Determination of Lung Lymphocyte Sub-populations

Monoclonal antibodies were all obtained from Becton Dickinson, Sunnyvale, CA. T cells were identified by the m noclonal antibody L u-4 (CD_3). Helper inducer T lymphocyte was recognized by monoclonal Leu-3. (CD4) and Suppr ssor

cytotoxic T subtype was identified by Leu-2 (CD8). Antibodies were FITC labeled. Unstained preparations were used as control to assess auto fluorescence and non relevant mouse IgG subclasses were used as controls for nonspecific binding. Stainings were done in microtiter plates by standard methods. Because alveolar macrophages tend to clump, each sample was diluted in 400 µl of staining buffer prior to analysis. Forward light scattering was used to analyze macrophages from lymphocytes first. Limiting gates were set on lymphocyte peak.

$$\% \text{ positive cells in gated population} = \frac{\text{Positive cells}}{\text{Total cells}} \times 100$$

Percentage of Leu-4$^+$ T cells in lymphocyte gates in patients with pulmonary sarcoid was > 90%. Helper to suppressor (or Leu3$^+$ to Leu2$^+$) ratio was also evaluated. In normal volunteers this T cell subtype ratio was approximately 2 similar to the ratio in blood.

Release of IL2 by Lung Mononuclear Cells

A fraction of lung mononuclear cells at a concentration of 10^6/ml was cultured in RPMI 1640 with 1% fetal Calf Serum for 48 hours. After this time period, supernatants were obtained by centrifugation and stored at -20°. Supernatants were assayed for the biochemical marker IL2 by their ability to stimulate H^3 thymidine incorporation in murine IL2 dependent CT-6 cells. Results are expressed as IL2 units by comparison with a standard. Quantitation uses profit analysis.

Analysis of Cell Proliferation

Lung lymphocyte replication was assessed by autoradiography. Lung mononuclear cells were incubated (10^6 cells/ml) in flat-bottom microliter wells in RPM I 1640 containing 10% heat-inactivated autologous serum and H^3 thymidine (0.5 µci, 2 ci/mmol, Amersham) for 24 hours. At this time, cytocentrifuge slide preparations were made of nonadherent cells. The cells were then fixed and extracted in methanol:acetic acid (3:1;v/v), developed for autoradiography with a ten day exposure and then stained

with Wright-Giemsa.

Lymphocyte labeling index = $\frac{\text{labeled lymphocyte}}{\text{total number of lymphocyte}} \times 100$

Study design and Patient Inclusion Criteria

Study included individuals on the following criteria:

1. Diagnosis of pulmonary sarcoidosis based on lung measurements including lung biopsy or mediastinal lymph node biopsy;

2. No therapy at initial evaluation (anti-inflammatory or immune suppressive);

3. Active lymphocytic alveolitis [(BAL data showing % lymphocytes $\geq$ 30% (normal $\pm$ 6%); Leu $^{3+}$: Leu $^{2+}$ $\geq$ 2.7 (normal 2$\pm$ 0.3); spontaneous IL2 level $\geq$ 5U/10^6 lung mononuclear cells (normal OU); spontaneous proliferation of lymphocytes in 24 hour $\geq$ 4% (normal ~ 1%)]. Of the 21 patients included in the study, ten were treated with steroidal formulations and all individuals were monitored every month up to six months. All were monitored for pulmonary function and the biochemical markers underlying the disease namely IL2 level and spontaneous proliferation of lymphocytes collected from patients.

Results obtained with Steroid Therapy

Lung lymphocyte levels, IL2 levels, lung helper to suppressor ratio and rate of lymphocyte proliferation over 24 hours period all decreased in patients treated with the steroidal formulations as shown in the Table X below:

Table X

Parameter Monitored	Initial Value %	Final Evaluation Untreated Group %	Final Evaluation Treated Group %
Lung lymphocytes %	52 ± 4	52 ± 5	34 ± 6%
Lung helper:Suppressor ratio	2.4 ± 0.5	7.8 ± 1.6	7.4 ± 0.8
Spontaneous IL2 ($U/2 \times 10^6$ cells)	14.6 ± 2.6	12.2 ± 2.3	0
Spontaneous T cell	6.7 ± 0.4	5.6 ± 0.8	< 1

WHAT IS CLAIMED IS:

1. A nonconventional liposome composition consisting essentially of nonphospholipid lipid components and a drug or salt or ester thereof.

2. The composition of Claim 1 wherein the lipid components are cholesterol and a salt of cholesterol ester wherein ester is selected from the group consisting of cholesterol sulfate, cholesterol phosphate, cholesterol stalate and cholesterol maleate and the salt is selected from the group consisting of sodium, potassium, lithium, magnesium and calcium.

3. The composition of Claim 2 wherein the cholesterol ester salt is sodium cholesterol sulfate.

4. The composition of Claim 3 wherein the ratio of sodium cholesterol sulfate to cholesterol to the drug is from 30 to 70 mole% of cholesterol sulfate, from 20 to 50 mole % of cholesterol, and from 0.1 to 20 mole % of the drug or salt or ester thereof.

5. The composition of Claim 4 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline,

norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines, hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

6. The composition of Claim 5 wherein the ratio of sodium cholesterol sulfate to cholesterol to the drug is 50:40:10.

7. The composition of Claim 5 wherein the ratio of sodium cholesterol sulfate to cholesterol to the drug is 55:40:5.

8. A method of treating allergic diseases by administering to a person in need of such treatment a therapeutically effective amount of nonconventional liposome composition consisting essentially of nonphospholipid lipid components and a drug or salt or ester thereof.

9. The method of Claim 8 wherein the lipid components are cholesterol ester salt and cholesterol in amounts from 30 to 70 mole % of cholesterol ester salt, from 20 to 50 mole % of cholesterol and from 0.1 to 20 mole % of the drug or salt or ester thereof.

10. The method of Claim 9 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxym rphone, hydr xyzines, hemophilus influ nza vaccine, pneumococcus vaccine, HIV

vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

11. The method of Claim 10, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 10.

12. The method of Claim 10, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of the drug of Claim 10.

13. A method of treating inflammatory diseases by administering to a person in need of such treatment a therapeutically effective amount of nonconventional liposome composition consisting essentially of nonphospholipid lipid components and a drug or salt or ester thereof.

14. The method of Claim 13 wherein the lipid components are cholesterol ester salt and cholesterol in amounts from 30 to 70 mole % of cholesterol ester salt, from 20 to 50 mole % of cholesterol and from 0.1 to 20 mole % of the drug.

15. The method of Claim 14 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline,

norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines, hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

16. The method of Claim 15, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 15.

17. The method of Claim 15, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of Claim 16.

18. A method of treating skin diseases by administering to a person in need of such treatment a therapeutically effective amount of topical nonconventional liposome composition consisting essentially of nonphospholipid lipid components and a drug or salt or ester thereof.

19. The method of Claim 18 wherein the lipid components are cholesterol ester salt and cholesterol in amounts from 30 to 70 mole % of cholesterol ester salt, from 20 to 50 mole % of cholesterol and from 0.1 to 20 mole % of the drug or salt or ester.

20. The method of Claim 19 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines,

hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

21. The method of Claim 20, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 20.

22. The method of Claim 20, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of the drug of Claim 20.

23. A method of treating arthritic or rheumatic diseases by administering to a person in need of such treatment a therapeutically effective amount of nonphospholipid liposome composition consisting essentially of nonphospholipid lipid components and a drug or salt or ester thereof.

24. The method of Claim 23 wherein the lipid components are cholesterol ester salt and from cholesterol in amounts from 30 to 70 mole % of sodium cholesterol sulfate, 20 to 50 mole % of cholesterol and from 0.1 to 20 mole % of the drug or salt or ester thereof.

25. The method of Claim 24 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisol ne, paramethasone, prednisol ne, prednisone, triamcinolone, metaproterenol sulfate, aminophylline,

terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines, hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

26. The method of Claim 25, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 25.

27. The method of Claim 25, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of the drug of Claim 25.

28. A method of suppressing neoplastic growth by administering to a person in need of such treatment a therapeutically effective am unt of nonconventional liposome composition comprising nonphospholipid lipids and a drug or

salt or ester thereof.

29. The method of Claim 28 wherein the lipid components are cholesterol ester salt and cholesterol in amounts from 30 to 70 mole % of sodium cholesterol sulfate, from 20 to 50 mole % of cholesterol and from 0.1 to 20 mole % of the drug or salt or ester thereof.

30. The method of Claim 29 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamin , pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interl ukin-

2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines, hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

31. The method of Claim 30, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 30.

32. The method of Claim 30, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of the drug of Claim 30.

33. A method of treating pulmonary diseases by administering via inhalation to the person in need of such treatment a therapeutically effective amount of nonconventional liposome composition consisting essentially of nonphospholipid lipid components and a steroidal drug or salts or esters thereof.

34. The method of Claim 33 wherein the lipid components are cholesterol ester salt and cholesterol in amounts from 30 to 70 mole % of sodium cholesterol sulfate, from 20 to 50 mole % of cholesterol and from 0.01 to 20 mole % of a drug or salt or ester thereof.

35. The method of Claim 34 wherein the drug is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, dexamethasone, difluorocortolone, fluclorolone, fluorocortisone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluorocortolone, fluorometholone, flurandrenolone, halcinonide, hydrocortisone, meprednisone,

methylprednisolone, paramethasone, prednisolone, prednisone, triamcinolone, metaproterenol sulfate, aminophylline, terbutaline, albuterol, theophyline, ephedrine, isoproterenol, bitolterol, pirbuterol, adrenaline, norepinephrine, procaterol, salmeterol, fluoromethasone, medrysone, fluticasone, atropine methyl nitrate, ipratropium bromide, cromolyn sodium, nedocromil, bleomycine, azathioprine, doxorubicin, daunorubicin, cyclophosphomide, vincristine, etoposide, lomustine, cisplatin, procarbazine, methotrexate, mitomycin, vindesine, ifosfamide, altretamine, acyclovir, azidothymidine, ganciclovir, enviroxime, ribavarin, rimantadine, amantadine, penicillin, erythromycin, tetracyclin, cephalothin, cefotaxime, carbenicillin, vancomycin, gentamycin, tobramycin, piperacillin, moxalactam, cefazolin, cefadroxil, cefoxitin, amikacin, amphotericin B, micozanole, apresoline, atenolol, captopril, verapamil, enalapril, dopamine, dextroamphetamine, pentamidine, pyribenzamine, chlorpheniramine, diphenhydramine, interferon, interleukin-2, monoclonal antibodies, gammaglobulin, ACTH, insulin, gonadotropin, dilaudid, demerol, oxymorphone, hydroxyzines, hemophilus influenza vaccine, pneumococcus vaccine, HIV vaccine and respiratory syncitial virus vaccine or their respective pharmaceutically acceptable salts or esters, alone or in combination.

36. The method of Claim 35, wherein the composition is 55 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 5 mole % of the drug of Claim 35.

37. The method of Claim 35, wherein the composition is 50 mole % of sodium cholesterol sulfate, 40 mole % of cholesterol and 10 mole % of the drug of Claim 35.

38. The method of Claim 35 wherein the pulmonary disease is interstilial lung disease.

39. A nonphospholipid lipid composition for treatment of interstitial lung diseases consisting essentially of nonphospholipid lipid component and a drug, or its salt or ester, suitable for delivery by inhalation into the deep lung wherein lipid component forms lipid particles.

40. The composition of Claim 39 wherein the lipid component is a mixture of cholesterol and a cholesterol ester salt and lipid particles are liposomes or micelles.

41. The composition of Claim 40 wherein the cholesterol ester is selected from the group consisting of sulfate, phosphate, nitrate and maleate and the salt is selected from the group consisting of sodium, potassium, lithium, magnesium and calcium.

42. The composition of Claim 40 wherein the ratio of sodium cholesterol sulfate to cholesterol to the drug is from 30 to 70 mole% of sodium cholesterol sulfate, from 20 to 50 mole % of cholesterol and from 0.01 to 20 mole % of the drug or the salt or ester thereof.

43. The composition of Claim 42 wherein the composition of Claim 42 is aerosolized into particles predominantly smaller than mass median aerodynamic diameter 2.1μ.

44. The composition of Claim 43 wherein the drug beclomethasone dipropionate present in amount between 0.4 to 2 mg/ml of liposome composition.

45. A process of preparing a suspension of nebulized aerosol particles of sizes predominantly smaller than 2.1 microns of nonphospholipid lipid particles

comprising:

(a) preparing a nonphospholipid lipid particles having sizes less than 1 micron in an aqueous suspension; and

(b) nebulizing suspension under conditions which produce aerosol particles of mass median aerodynamic diameter predominantly smaller than 2.1 microns.

46. The process of Claim 45 wherein the lipid particle is liposome.

47. The process of Claim 45 wherein the lipid particle is micelle.

48. The process of Claim 45 wherein the nebulizer is any nebulizer suitable for the generation of particle aerosols predominantly smaller than 2.1 microns mass median aerodynamic diameter.

49. A nonphospholipid micelle composition for treatment of interstitial lung diseases consisting essentially of nonphospholipid lipid components and a drug or its salt or ester, suitable for delivery by inhalation into the deep lung, wherein micelle sizes are predominantly not larger than 0.2 microns.

50. A nonphospholipid liposome composition for treatment of interstitial lung diseases consisting essentially of nonphospholipid lipid components and a drug or its salt or ester, suitable for delivery by inhalation into the deep lung wherein liposome sizes are predominantly not larger than 2.1 microns.

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OF INTERNATIONAL PROCESSING

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IN RNATIONAL SEARCH REPORT

International Application No. PCT/US89/05525

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 43/00 49/00, 37/22
U.S. CL. 424/1.1, 9, 450

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/1.1, 9, 450

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,693,999 (AXGLSSON ET AL.) 15 SEPTEMBER 1987 SEE THE ENTIRE DOCUMENT.	1-50
Y	US, A, 4,780,455 (LIEBERMAN ET AL.) 25 OCTOBER 1988 SEE THE ENTIRE DOCUMENT.	1-50
A	US, A, 4,235,871 (PAPAHADJOPOULOS ET AL.) 25 NOVEMBER 1980 SEE THE ENTIRE DOCUMENT.	1-50
A	US, A, 4,515,736 (DEAMER) 7 MAY 1985 SEE THE ENTIRE DOCUMENT.	1-50

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 JANUARY 1990	27 FEB 1990

International Searching Authority	Signature of Authorized Officer
ISA/US	PENNY PRATER

Form PCT/ISA/210 (second sheet) (Rev.11-87)